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Neuroethology of the colonial mind: Ecological and evolutionary context of social brains

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Neuroethology of the colonial mind: Ecological and evolutionary context of social brains

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Editorial: Neuroethology of the colonial mind: Ecological and evolutionary context of social brains

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Editorial on the Research Topic
Neuroethology of the colonial mind: Ecological and evolutionary context of social brains

Collective behavior relies on interactions among individuals who have neural substrates supporting the exchange and processing of social information (Gordon, 2021). The collective acquisition and processing of information in animal groups suggest that individuals form a "colonial mind." Over the past decades, studies of individual and collective cognition have received a lot of attention (Couzin, 2009; Simons and Tibbetts, 2019). However, little is known about how the two systems interact. For instance, while collective cognition necessarily emerges from individual cognition, individual cognitive abilities are not correlated to collective cognitive abilities (Feinerman and Korman, 2017). Studying cognitive processes across levels of biological organization thus requires a better understanding of the mechanisms of cognition at each level and within an evolutionary context. This necessitates analyzing how animals use social information in different contexts or understanding the neural adaptations associated with group living and ecological challenges. For this research topic, we brought together researchers in neuroscience and collective animal behavior to further examine these aspects of the colonial mind.

Social information transfer can yield fitness benefits to individuals (Krause et al., 2010). For instance, grouped animals often respond faster and more accurately to changes in environmental or social circumstances than isolated conspecifics (Sumpter, 2010). While these cognitive advantages were long considered exclusive to the most socially advanced animals, recent studies show collective cognition can be beneficial across the animal kingdom, even in loosely social species. For instance, Mörchen et al. demonstrate that orangutans, which are less social than other apes, learn about new environments through social information gathered from local individuals when migrating. Ferreira et al. showed how social information influences individual reactions in gregarious fruit flies under threatening situations.

By contrast, the mechanisms underlying the transition from solitary to group living are much less understood. Several physiological changes may have contributed to division of labor in highly social species. Sasaki et al. provide a comparative perspective to understand how neurotransmitters and hormones evolved to support eusociality. The authors compiled literature from eusocial and non-social insects and provided support for the "ovarian ground plan hypothesis" (Amdam et al., 2004), suggesting ovarian function and behavior is physiologically separated into reproductive and non-reproductive forms. They also provided support for the "split-function hypothesis" (West-Eberhard, 1996), which proposes that juvenile hormone, ancestrally involved in reproduction, evolved to have an additional role in worker division of labor (Sasaki et al.).

Neuroanatomical comparisons can also inform researchers about how animals transitioned from solitary to social life. The "social brain hypothesis" posits that increasing levels of sociality are associated with larger brains to support the processing of more social information (Dunbar, 1998). However, the distributed cognition of highly integrated groups with division of labor may alleviate the cognitive load on the individuals, and potentially reduce their neural requirements (Gronenberg and Riveros, 2009). Testing this hypothesis in eusocial insects has so far produced mixed results (O'Donnell et al., 2015, 2019; Kamhi et al., 2016; Sayol et al., 2020). The variation in behaviors and life histories that characterize sociality may be part of the reason for the inconsistencies observed. For instance, many socio-cognitive behaviors once thought to be specific to social species, such as the recognition of individual identity or social learning, have recently been described in non-social animals and may be primarily related to foraging and mating (Poissonnier et al.). Researchers therefore should be more selective in the behaviors associated with sociality in comparative studies.

Accordingly, several studies began to focus on specific characteristics of sociality to better understand how the brain evolves to support particular social behaviors. For example, Caponera et al. defined five characteristics of sociality (intragroup competition, relationship differentiation, information sharing, dominance hierarchies, and task specialization and redundancy) and included an example of how to apply these criteria in a comparison of social and subsocial spiders. The authors found that task redundancy in social spiders was correlated with a reduction in the arcuate body, a brain region involved in mechanosensory integration (Steinhoff et al., 2017). In a similar analysis, Godfrey et al. showed that differences in olfactory processing regions associated with nestmate recognition are positively correlated with colony size across Leptomyrmecini ant species. As in the social brain hypothesis, the authors proposed that increased colony size is associated with a greater need for nestmate recognition; however, they focused specifically on the circuitry that supports this behavior.

Increased investment in olfactory processing is also associated with nestmate interactions in the social wasp *Polistes dominula* (Gandia et al.). In this species, females have larger antennal lobes, while males have larger optic lobes, the primary olfactory and visual processing regions, respectively (Gronenberg, 2008). These differential neural investments correspond to the importance of social interactions in group living for females and the reliance on vision in mating for males. Similarly, reproductive females may have a greater need for group interactions than female workers that primarily forage, and had larger mushroom bodies, a region involved in higher order sensory integration (Fahrbach, 2006). Thus, it is important to account for variation in behavior within the social group. Brain region size of individuals within groups appears to have adapted to the sensory requirements associated with the individuals' specific behavioral requirements (e.g., Arganda et al., 2020).

While social context undoubtedly shapes cognitive and neural function, sociality exists within a broader context of the environment, which also may affect neural circuitry (Healy, 2021). Non-social behaviors such as navigation (Sayol et al., 2020) and foraging (Farris and Roberts, 2005; Farris, 2008; Sheehan et al., 2019) have been shown to influence neural investment. Azorsa et al. used the "ecological brain hypothesis," which states that the brain evolves to account for the cognitive challenges associated with foraging and processing food (DeCasien et al., 2017; Lihoreau et al., 2019; Simons and Tibbetts, 2019), to discuss how predation foraging ecology may interact with group living to affect the sensory requirements and cognitive processing of the species.

Comparative analyses of brain size in relation to the socio-ecology of social insects can provide insight for findings in other organisms. Through a study of fossil records in early humans, DeSilva et al. suggest that there has been a recent decrease in brain size in humans. Using observations from comparative studies of ant neuroanatomy, these authors propose that the trend they observed may be associated with characteristics of collective behavior such as increased sociality, sharing of information, and group decision-making (but see comment by Villmoare and Grabowski and response by DeSilva et al. about potential issues of using rare fossil records for such analyses).

The studies in this collection seek to understand how behavioral and neural characteristics enable individuals to engage in social behaviors and how social organization, or collective behavior, may alter individual cognition. They take advantage of the approaches previously mentioned to nicely illustrate how research on the evolution of brains and cognition has recently moved from broad correlations between brain sizes and social organization (Dujardin, 1850; Dunbar, 1998) to more detailed considerations of the neuroethology of specific socio-cognitive behaviors (Lihoreau et al., 2012; Godfrey and Gronenberg, 2019). Future research will have to account for variation in cognition across group members (Naug and Tait) and life history strategies that characterize the group.

Recent studies, including those in this collection, have progressed our understanding of the neural underpinnings of collective cognition, but more can still be done. While the diversity of animal models used in collective cognition is increasing (i.e. social insects, *Drosophila*, primates), an important effort should be made to broaden the scope further with species comparisons across the spectrum of social organization. The application and tuning of tools such as statistical brain atlases (Arganda et al.) will make these large-scale comparative studies feasible and accurate. Ultimately, a better understanding of the neurobiology of collective minds across the animal kingdom, including humans, may be useful for developing more efficient collective decisions, more robust artificial systems (e.g., Ebert et al., 2020), and more informed interactions with wildlife.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Social Evolution With Decoupling of Multiple Roles of Biogenic Amines Into Different Phenotypes in Hymenoptera

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Convergent evolution of eusociality with the division of reproduction and its plastic transition in Hymenoptera has long attracted the attention of researchers. To explain the evolutionary scenario of the reproductive division of labor, several hypotheses had been proposed. Among these, we focus on the most basic concepts, i.e., the ovarian ground plan hypothesis (OGPH) and the split-function hypothesis (SFH). The OGPH assumes the physiological decoupling of ovarian cycles and behavior into reproductive and non-reproductive individuals, whereas the SFH assumes that the ancestral reproductive function of juvenile hormone (JH) became split into a dual function. Here, we review recent progress in the understanding of the neurohormonal regulation of reproduction and social behavior in eusocial hymenopterans, with an emphasis on biogenic amines. Biogenic amines are key substances involved in the switching of reproductive physiology and modulation of social behaviors. Dopamine has a pivotal role in the formation of reproductive skew irrespective of the social system, whereas octopamine and serotonin contribute largely to non-reproductive social behaviors. These decoupling roles of biogenic amines are seen in the life cycle of a single female in a solitary species, supporting OGPH. JH promotes reproduction with dopamine function in primitively eusocial species, whereas it regulates non-reproductive social behaviors with octopamine function in advanced eusocial species. The signal transduction networks between JH and the biogenic amines have been rewired in advanced eusocial species, which could regulate reproduction in response to various social stimuli independently of JH action.

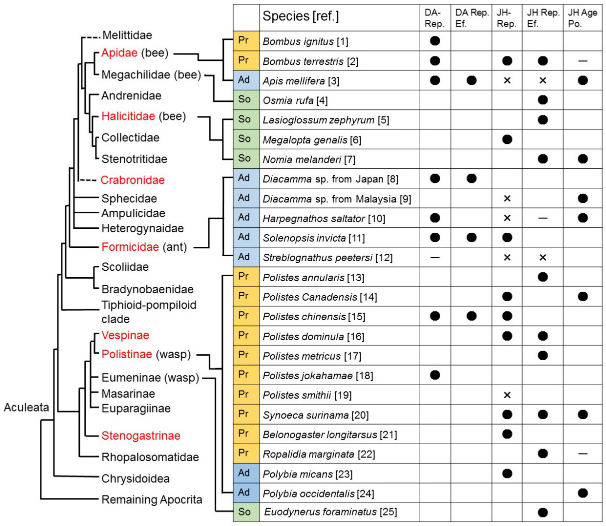
Keywords: biogenic amine, division of labor, eusociality, Hymenoptera, reproduction, social evolution

INTRODUCTION

Eusociality in insects, characterized by sophisticated division of labor among group members, is assumed to have evolved convergently from non-eusocial ancestors in an individual taxon such as Hymenoptera (bees, wasps, and ants), Blattodea (termites), Hemiptera (aphids), Thysanoptera (thrips), and Coleoptera (beetles) (reviewed by Berens et al., 2015; Toth and Rehan, 2017; Costa, 2018). The insect eusociality is defined by cooperative brood care, overlapping generations between the parent and adult offspring, and a reproductive division of labor (castes) (Wilson, 1971). Based on the stability of castes and the degree of morphological dimorphism between castes, eusociality in Hymenoptera is further divided into three groups: facultative, primitive and advanced eusociality (Hunt, 2012). Facultative eusociality is characterized by a flexible presence in the non-morphological castes. In primitive eusociality, the non-morphological castes are always present, and behaviorally distinct and plastic, whereas advanced eusocial organisms with rigid morphological castes probably originated from primitive eusociality (Hunt, 2012; Jandt and Toth, 2015; Toth and Rehan, 2017).

To explain the evolutionary process of eusociality from solitary to advanced eusociality in Hymenoptera, several mechanistic hypotheses for social evolution have been proposed (West-Eberhard, 1996; Robinson and Vargo, 1997; Hunt, 2007; Jandt and Toth, 2015). We introduce two evolutionary hypotheses involved in reproductive physiology. First is the ovarian ground plan hypothesis (OGPH), which focuses on the physiological decoupling of ovarian cycles and behavior into reproductive and non-reproductive states (West-Eberhard, 1996). This hypothesis comprises three components. The first is a proposed cycle of ovarian activity in a solitary ancestor of the social species. The second is the context-dependent expression of alternative behaviors from a single genotype (phenotypic plasticity) that can occur among females that nest together, each with a solitary ovarian ground plan. The third is the evolution of a switch-like mechanism that regulates phenotypic expression into two castes: queens and workers (West-Eberhard, 1996; Hunt, 2007). This hypothesis predicts that physiological characteristics related to caste-specific behavior in eusocial species are similar to those of reproductive and non-reproductive behavior in a single female in a solitary species (West-Eberhard, 1996; Jandt and Toth, 2015; Trumbo, 2018). A related hypothesis termed the reproductive ground plan hypothesis (RGPH) posits that the regulation of the division of labor among workers arose from a further split of solitary gene networks related to reproduction (Amdam et al., 2004; Amdam and Page, 2010). The present paper discusses the evolutionary process of the reproductive division of labor from solitary to advanced eusociality, which is more related to OGPH than RGPH.

Second is the split-function hypothesis (SFH) that focuses on the function of juvenile hormone (JH) (West-Eberhard, 1996; Robinson and Vargo, 1997; Jandt and Toth, 2015). JH is a hemolymphatic hormone released from corpora allata and is well known as a key factor regulating the female reproductive state in solitary insects (Nijhout, 1994; Hartfelder, 2000; Raikhel et al., 2005; Jindra et al., 2013). In primitive eusocial Hymenoptera, including paper wasps and bumble bees, JH promotes ovarian development and egg-laying behavior as well as solitary species (Robinson and Vargo, 1997; Tibbetts et al., 2011a; Shpigler et al., 2014; **Figure 1**). In contrast, in advanced eusocial species, including honey bees and ants, high JH titer is associated with an age-related behavioral shift from nursing to foraging (Robinson and Vargo, 1997; Bloch et al., 2009; **Figure 1**). These findings lead to the suggestion that JH function has shifted from a gonadotropin to a behavioral regulator in workers during the social evolution. The SFH assumes that the ancestral reproductive function of JH became split into a dual function, regulating reproduction in queens and behavioral division of labor among workers (i.e., nest construction, nursing, defense, and foraging), and in advanced eusocial species, the reproductive function of JH might have been lost. Therefore,



	Species [ref.]	DA-Rep.	DA Rep. Ef.	JH-Rep.	JH Rep. Ef.	JH Age Po.
Pr	*Bombus ignitus* [1]	●				
Pr	*Bombus terrestris* [2]	●		●	●	–
Ad	*Apis mellifera* [3]	●	●	×	×	●
So	*Osmia rufa* [4]				●	
So	*Lasioglossum zephyrum* [5]				●	
So	*Megalopta genalis* [6]			●		
So	*Nomia melanderi* [7]				●	●
Ad	*Diacamma* sp. from Japan [8]	●	●			
Ad	*Diacamma* sp. from Malaysia [9]			×		●
Ad	*Harpegnathos saltator* [10]	●		×	–	●
Ad	*Solenopsis invicta* [11]	●	●	●		
Ad	*Streblognathus peetersi* [12]	–		×	×	
Pr	*Polistes annularis* [13]				●	
Pr	*Polistes Canadensis* [14]			●		●
Pr	*Polistes chinensis* [15]	●	●	●		
Pr	*Polistes dominula* [16]			●	●	
Pr	*Polistes metricus* [17]				●	
Pr	*Polistes jokahamae* [18]	●				
Pr	*Polistes smithii* [19]			×		
Pr	*Synoeca surinama* [20]			●	●	●
Pr	*Belonogaster longitarsus* [21]			●		
Pr	*Ropalidia marginata* [22]				●	–
Ad	*Polybia micans* [23]			●		
Ad	*Polybia occidentalis* [24]					●
So	*Euodynerus foraminatus* [25]				●	

FIGURE 1 | Roles of dopamine and juvenile hormone in reproductive females in Hymenoptera. A phylogenetic tree based on Gullan and Cranston (2014) is modified. Possible non-monophyletic families are shown in quotes on a dashed branch. Families containing eusocial species are indicated in red. In this figure, evidence of correlation and causal relationships are extensively evaluated. Correlation between dopamine levels in the brains and reproduction (DA-Rep.), and between juvenile hormone titers in hemolymph and reproduction (JH-Rep.) are indicated as positive (•), negative (×), and neutral (–). Effects of dopamine (DA Rep. Ef.) and juvenile hormone (JH Rep. Ef.) on reproduction, the relation of juvenile hormone with age polyphenism (JH Age Po.) are also indicated. Ad: advanced eusocial, Pr: primitively eusocial, So: solitary and facultatively eusocial (Bohm, 1972; Bell, 1973; Barth et al., 1975; Röseler, 1977; Robinson et al., 1992; O'Donnell and Jeanne, 1993; Sommer et al., 1993; Harris and Woodring, 1995; Robinson and Vargo, 1997; Bloch et al., 2000; Pinto et al., 2000; Boulay et al., 2001; Sasaki and Harada, 2020; Agrahari and Gadagkar, 2003; Brent and Vargo, 2003; Dombroski et al., 2003; Cuvillier-Hot et al., 2004; Giray et al., 2005; Brent et al., 2006; Cuvillier-Hot and Lenoir, 2006; Sasaki et al., 2007, 2009; Penick et al., 2011; Tibbetts et al., 2011b; Wasieleski et al., 2011; Tibbetts et al., 2013a; Smith et al., 2013; Amsalem et al., 2014; Kelstrup et al., 2014a; Kelstrup et al., 2014b; Penick et al., 2014; Shpigler et al., 2014; Kelstrup et al., 2015, 2017; Okada et al., 2015; Kapheim and Johnson, 2017; Sasaki et al., 2017; Tsuchida et al., 2020; Yoshimura et al., 2021).

this hypothesis predicts that JH regulates reproduction only in solitary species, and regulates both reproduction and division of labor among workers in primitive eusocial species (West-Eberhard, 1996; Jandt and Toth, 2015).

To re-evaluate the SFH and OGPH, we focus on the substances that directly regulate neural activities for social behavior. Social behavior is largely regulated by several types of interaction among colony members (e.g., dominance interactions, pheromones, or trophallaxis). These stimuli are neurally inputted as multimodal sensory signals and processed in the central nervous system (CNS). The behavioral outputs are adaptively plastic, depending on the colony status. Therefore, the neural process–behavioral output pathway might not be as fixed as expected, but instead be flexibly selected from available multiple pathways or combined multiple pathways in the CNS by neural modifiers. One such group of neural modifiers is the biogenic amines. Biogenic amines are neuroactive substances that elicit certain behaviors and physiological states and are widely conserved among vertebrates and invertebrates. They have roles as neurotransmitters (acting on a synapse), neuromodulators (acting on local neural circuits), and neurohormones (hormonal function in remote tissues) in both the peripheral nervous system and CNS (Evans, 1980; Roeder, 2005; Lange, 2009). Thus, they are probably the fundamental factors mediating social interactions and the output of social behaviors.

Recently, there has been a significant increase in the number of physiological studies on social behaviors (especially in social hymenopterans) and the results have suggested the regulatory mechanisms of biogenic amines together with roles of JH and environmental factors. Therefore, here we integrate recent data on biogenic amines and other factors to highlight connections between biogenic amines and social behavior in eusocial hymenopterans, leading to an improved understanding of social evolution.

REPRODUCTIVE DIVISION OF LABOR AND WORKER REPRODUCTION

The reproductive division of labor is fundamental to the organization and evolution of insect societies. In primitive eusocial hymenopterans, the caste fate can be mainly determined at the adult stage, with a pre-imaginal bias generated at the larval stage (O'Donnell, 1998; Berens et al., 2015). In *Polistes* wasps, the cues related to caste determination during the pre-imaginal stage include levels of larval nutrition and frequency of vibrational stimuli (O'Donnell, 1998; Suryanarayanan et al., 2011). The cues during the adult stage are the presence of a queen and photoperiods (Bohm, 1972; Solís and Strassmann, 1990; Yoshimura et al., 2021).

In advanced eusocial societies, including honey bees, stingless bees, and most ants, reproduction is strongly biased toward queens. Queens have a high reproductive ability relative to workers, which are either sterile or have low reproductive potential. Caste-specific developmental pathways depending on the nutritional condition during the larval stages might, to some degree, result in two adult phenotypes (Wilde and Beetsma, 1982; Asencot and Lensky, 1988; Kamakura, 2011; Leimer et al., 2012; Corona et al., 2016) and a specialized adult brain that is morphologically and physiologically adapted to the performance of caste-specific behavioral tasks (Snodgrass, 1956; Michener, 1974; Arnold et al., 1988; Groh and Rössler, 2008). How the caste-specific behaviors are physiologically regulated is an important issue in the reproductive division of labor in social insects. The details of such caste-specific physiology would provide us with a key to further our understanding of social evolution.

Reproductive Function of JH

In primitive eusocial Hymenoptera, JH promotes ovarian development and egg-laying behavior at adult stages as well as in solitary species (Raikhel et al., 2005; Tibbetts et al., 2011a; Shpigler et al., 2014). Whereas, in advanced eusocial species, JH inhibits the synthesis of a precursor of egg yolk vitellogenin or does not affect ovarian development (Robinson and Vargo, 1997; Pinto et al., 2000; Bloch et al., 2009; **Figure 1**). These findings well fit the SFH that the ancestral reproductive function of JH became split into a dual function, regulating reproduction in queens and behavioral division of labor among workers. In contrast, this evidence does not support the OGPH, because JH reproductive function is no longer conserved in reproductive individuals in advanced eusocial species. In advanced eusocial species, it is expected that other substances instead of JH may drive cascades of ovarian development for reproduction.

Caste-Specific Behaviors Mediated by Biogenic Amines

Queen-worker differences in dopamine levels in the brain have been reported in several species including the bumble bee *Bombus ignitus* (Sasaki et al., 2021) and the honey bee *Apis mellifera* (Brandes et al., 1990; Sasaki et al., 2012, 2018), but not found in some ants (e.g., *Formica japonica*, Aonuma and Watanabe, 2012a). In the bumble bee, the dopamine levels in the brains of emerged queens (gynes) are approximately two times higher than in emerged workers (Sasaki et al., 2021). The levels of the precursor and metabolite of dopamine are also higher in queens, suggesting the upregulation of the synthetic pathway of dopamine in the brain of the queen. In the honey bee, the dopamine levels in the brains of virgin queens were 3.5–7 times higher than that in the same aged workers (Sasaki et al., 2012, 2018). The caste differences in dopamine levels in the brain occur during pupal stages with upregulation of gene expression of enzymes involved in dopamine biosynthesis (Sasaki et al., 2018). From the larval stage to the adult stage, nurse bees provide queens with food known as "royal jelly," which contains the dopamine precursor tyrosine (Haydak, 1970; Liming et al., 2009). Artificial feeding of royal jelly during the larval stage increases tyrosine flow with an elevation of catecholamines including dopamine in the brain of queen-like adult females (Sasaki and Harada, 2020).

In honey bees, the high levels of dopamine in both brain and hemolymph in virgin queens can contribute to the enhancement of fighting with rival nestmate queens (Farkhary et al., 2017; Sasaki and Harada, 2020), locomotor activities (Harano et al., 2008), and flight activities (Farkhary et al., 2019), which could lead to active mating flight. In the fire ant, *Solenopsis invicta*, virgin queens shed their wings (dealation) and lay unfertilized male eggs in response to their isolation from nestmates. The isolated virgin queens have higher dopamine levels in their brains than the same aged non-isolated virgin queens (Boulay et al., 2001). The inhibition of tyrosine hydroxylase for dopamine biosynthesis reduced egg production, whereas restoring dopamine biosynthesis with a dopamine precursor restored oogenesis and oviposition, suggesting that dopamine promotes reproduction (Boulay et al., 2001). Thus, dopaminergic activities seem to be one of the conserved physiological characteristics in the brain of the reproductive caste in bees and ants, although more evidence is required in other species across multiple levels of eusociality.

Worker Reproduction and Dominance Hierarchy Among Females

In many advanced eusocial hymenopterans, workers cannot mate despite retaining functional ovaries and producing unfertilized eggs that develop parthenogenetically into males. Although workers are usually infertile with inactivated ovaries in the presence of a queen, worker reproduction frequently occurs in the absence of queens. Even in facultative and primitive eusocial species, subordinate reproduction can be suppressed behaviorally and pheromonally by dominant females in a similar way to the suppression of worker reproduction in advanced eusocial species. Recent literature suggests consistently that brain dopamine is involved in the behavioral and physiological transitions of non-reproductives to reproductives in several lineages (**Figures 1**, **2**). Dopamine levels in the brains are positively correlated with the reproductive states of workers in bumble bees (*B. terrestris*: Bloch et al., 2000; *B. ignitus*: Sasaki et al., 2017), paper wasps (*Polistes chinensis*: Sasaki et al., 2007; *Polistes jokahamae*: Yoshimura et al., 2021), the honey bee *A. mellifera* (Harris and Woodring, 1995; Sasaki and Nagao, 2001), and reproductive females (gamergates) in ant species that retain totipotent workers (*Harpegnathos saltator,* Penick et al., 2014; *Diacamma* sp., Okada et al., 2015; **Figure 1**). Experimental manipulations using dopamine suggested gonadotropic functions and activation of ovaries in workers of *A. mellifera* (Dombroski et al., 2003), *P. chinensis* (Sasaki et al., 2009), and *Diacamma* sp. (Okada et al., 2015). The combination of this correlative evidence with the experimental evidence suggests that the reproductive function of dopamine is shared among several species in multiple eusocial lineages in Hymenoptera.

The gene expression of dopamine receptors in the brains or ovaries of reproductive workers can be influenced by queen substances in *A. mellifera* (Beggs et al., 2007; Vergoz et al., 2012) and by isolation from interactions with nestmates in *H. saltator* (Penick et al., 2014) and *Diacamma* sp. (Okada et al., 2015; Shimoji et al., 2017; **Figure 3**). In the honey bee, two dopamine receptor genes, *Amdop1* (DopR1) and *Amdop2* (DopR2) decrease in the brains of reproductive workers (Vergoz et al., 2012; **Figure 3**). Since the expression of *Amdop1* and *Amdop2* in the brains of virgin queens are lower than in normal workers (Sasaki et al., 2018), the downregulation of these genes in reproductive workers suggests a transition to a queen-like state. In a monomorphic ant *Diacamma*, D1-like (human dopamine receptor subtype 1-like) receptor genes (*dopr1* and *dopr2*) are abundant in fat bodies of gamergates (Okada et al., 2015), suggesting that the fat body is a potential target of dopamine to stimulate vitellogenin (Vg) synthesis for ovarian activation (**Figure 3**). Thus, the expression of dopamine receptors in the brains and peripheral organs involved in ovarian development supports the gonadotropic neurohormonal function of dopamine.

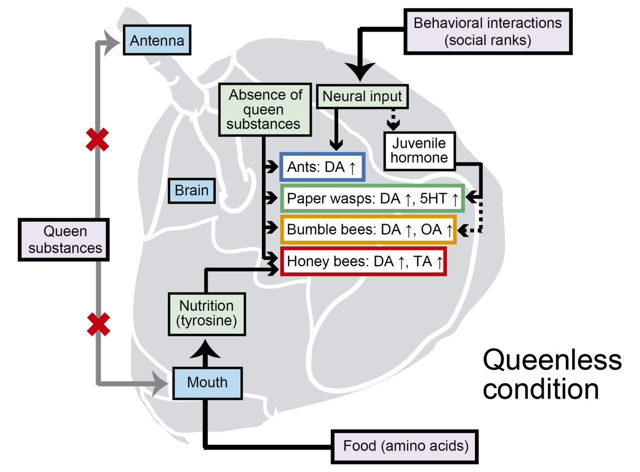


FIGURE 2 | Factors affecting increases in biogenic amines in the brain during the transition of non-reproductives to reproductives in Hymenoptera. Purple boxes indicate environmental factors. Dashed lines indicate hypothetical pathways. DA: dopamine, OA: octopamine, TA: tyramine, 5HT: serotonin. In this summary, "queen" and "queenless" are used in a broad sense to include functional queens in ants (i.e., gamergates).

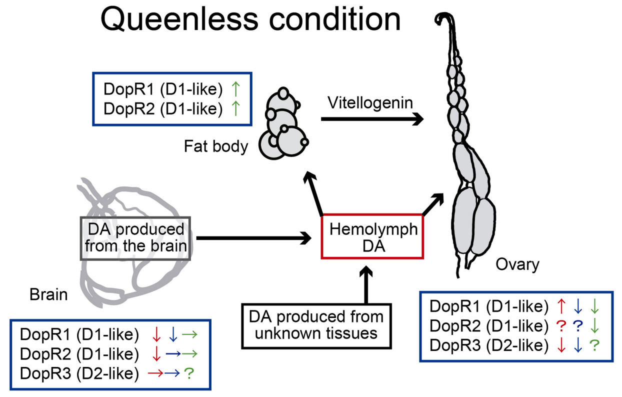


FIGURE 3 | Gene expression of dopamine receptors in the brain and peripheral tissues of reproductive workers. Red: *Apis mellifera*, blue: *Harpegnathos saltator*, green: *Diacamma.* sp. The expression levels of receptor genes are based on Vergoz et al. (2012); Penick et al. (2014), and Okada et al. (2015). In this summary, "queen" and "queenless" are used in a broad sense to include functional queens in ants (i.e., gamergates).

Although dopamine has a predominant gonadotropic function across taxa, brain tyramine might also promote reproduction with dopamine in workers in the honey bee. The levels of brain tyramine in worker honey bees increase with dopamine in response to the removal of a queen (Sasaki and Nagao, 2002). The oral application of tyramine can inhibit flying for foraging (Schulz and Robinson, 2001; Fussenecker et al., 2006) and promote ovarian activity (Sasaki and Harano, 2007) and the production of queen-like pheromones by Dufour's gland and the mandibular glands (Salomon et al., 2012). In the paper wasp *P. jokahamae*, the long-day photoperiod condition increases the tyramine level in the brain and causes the initiation of ovarian development (Yoshimura et al., 2021). In this paper wasp, the levels of tyramine and dopamine in the brains are correlated with ovarian development (Yoshimura et al., 2021). Thus, mediation by both dopamine and tyramine, which have a common precursor tyrosine, suggests the involvement of tyrosine in ovarian activation. Later in this article, we focus on current advances in understanding the environmental and physiological regulation of dopamine, as a pivotal orchestrator of reproductive physiology and behavior.

Social Factors Affecting Dopamine Levels in the Brain

Determining the regulatory mechanisms underlying an increase in brain dopamine depending on the social environment is important for understanding the regulation of reproduction in eusocial species. Several pioneering studies have begun to elucidate various environmental and social factors involved in the regulatory mechanisms (Beggs et al., 2007; Penick et al., 2014; Matsuyama et al., 2015; Shimoji et al., 2017). Behavioral interactions (dominance interaction), pheromonal signals (queen substances), and nutrition (food intake) are plausible candidates in the influence of brain dopamine levels that control reproductive states (**Figure 2**).

Dominance Interaction

In behavioral interactions, aggressive behavior can influence reproductive states among females. In primitive eusocial species, social ranks including pre-contest assessment behavior and reproductive states are associated with JH titer (Röseler, 1977; Giray et al., 2005; Tibbetts et al., 2013b; Tsuchida et al., 2020). Given that dopamine is also related to reproductive states in primitive eusocial species (Bloch et al., 2000; Sasaki et al., 2007, 2017; Yoshimura et al., 2021), a relationship between JH and dopamine is expected. In fact, the application of JH can enhance the dopamine levels in the brain of workers in the paper wasp *P. chinensis* (Tsuchida et al., 2020). In contrast, the reproductive roles of JH have been lost in several advanced eusocial species (**Figure 1**; Robinson and Vargo, 1997; Bloch et al., 2009), such that other substances downstream of JH, including dopamine, might have a function in promoting reproduction.

In the ant *H. saltator*, after being subjected to aggressive behaviors by nestmates, workers have lower dopamine levels in their brains compared with those nestmates (Penick et al., 2014). In *Diacamma* sp., dominant workers that aggress other workers have higher dopamine levels in their brains compared with lower-ranked subordinate workers (Shimoji et al., 2017). Such subordinate workers have elevated levels of brain dopamine after isolation from the dominant workers and thus are no longer subjected to attack. These observations suggest that dopamine in the brain is regulated by aggressive behavior (Shimoji et al., 2017; **Figure 2**). The level of dopamine increases in the brain of the winner of an aggressive interaction to activate her ovaries, whereas the loser decreases the level of brain dopamine to suppress ovarian activation. If the brain dopamine level or the ovarian activity positively influences their aggressiveness, a positive feedback process can finally lead to the monopolization of reproduction by a particular female. However, physiological factors determining the level of aggressiveness in dominance interactions are yet to be investigated.

Queen Substances

Queen substances can control worker behavior and physiology in advanced eusocial bees as pheromonal signals. In the honey bee, a queen pheromone, homovanillyl alcohol (HVA), can reduce brain dopamine levels (Beggs et al., 2007). HVA has the potential to bind a dopamine receptor (AmDOP3) and to act as an agonist (Beggs and Mercer, 2009). HVA can control brain dopamine in two ways: the first is the detection of HVA by antennae and the resulting transmission of neural signals to influence brain dopamine; the second is via the oral intake of HVA so that it can act directly on the dopaminergic systems in the brain via the hemolymph (**Figure 2**). The former is supported by results showing the neural processing of chemosensory signals from queen substances in the antennal lobes (Carcaud et al., 2015). Another component of queen substances in the honey bee, (2E)-9-oxodecenoic acid (9ODA), also has important functions to inhibit ovarian activation in workers and is detected by antennae with particular odorant receptors and transduced as neural signals (Wanner et al., 2007). Although novel, the latter mechanism requires more evidence. Given that other chemicals (e.g., cuticular hydrocarbons) that can be sensed by antennae are used as queen substances in other hymenopteran species (e.g., Van Oystaeyen et al., 2014), the detection of queen substances by the antennae might be a common pathway (d'Ettorre et al., 2004), whereas the oral intake of queen substances might be an additional mechanism in the honey bee.

Food Intake

Food consumption is influenced by the presence of a queen and her brood in a colony and affects the supply of precursors of biogenic amines. In the honey bee, royal jelly contains tyrosine, which is a common precursor of dopamine, tyramine, and octopamine, although tyrosine is not the most abundant of the 26 amino acids that are contained in royal jelly (Townsend and Lucas, 1940; Haydak, 1970; Liming et al., 2009). Royal jelly is normally fed by nurse bees to the queen and larvae in the queenright colony, whereas, in queenless colonies without broods, it can be shared among nurse bees that can become reproductive individuals. Therefore, reproductive individuals in queenless colonies might ingest a relatively large amount of tyrosine by consuming royal jelly-like food. This intake of tyrosine can enhance the levels of brain dopamine and tyramine in queenless workers and accelerate their transition from normal workers to reproductive individuals (Matsuyama et al., 2015; **Figure 2**). This mechanism might operate not only in honey bees but also other social insects in which the brain dopamine levels either increase slowly in the absence of a queen or are maintained at a high level during reproduction. These species require nutrition for ovarian activation, with supplies of dopamine precursors. Thus, dopamine metabolism and signaling appear to have a pivotal role in the formation of reproductive skew across various social systems, i.e., from primitive societies in which physical interaction among monomorphic females regulates the reproductive division of labor to advanced societies where pheromones control it remotely.

Reproductive Function of Dopamine in Solitary Insects

The reproductive function of dopamine in solitary insects is a prerequisite for assuming dopamine as the core physiological system in OGPH. The OGPH assumes that ancestral solitary species possess functional aminergic systems for both reproductive and non-reproductive activities. The OGPH predicts that one functional system for reproduction expresses in reproductive females, the other non-reproductive system occurs in workers. Reproductive females in primitive and advanced eusocial hymenopterans seem to possess the dopaminergic system for reproduction as mentioned above.

Reproductive functions of the dopamine in females of solitary insects have been reported in Blattodea (cockroach *Blattella germanica*: Pastor et al., 1991), Hemiptera (linden bug *Pyrrhocoris apterus*: Chvalova et al., 2014; plant bug *Lygus hesperus*: Brent et al., 2016), Coleoptera (red flour beetle *Tribolium castaneum*: Bai and Palli, 2016), and Diptera (fruit fly *Drosophila melanogaster*: Neckameyer, 1996; Pendleton et al., 1996; mosquito *Anopheles gambiae*: Fuchs et al., 2014). In these species, dopamine accelerates ovarian development and egg-laying behavior, often in a nutrition-dependent manner. In *A. gambiae*, the inhibition of tyrosine (a precursor of dopamine) supply causes a decrease in oviposition rate, fecundity, and egg hatching rate (Fuchs et al., 2014). In *T. castaneum*, the knockdown of the dopamine D2-like receptor gene leads to a reduction in vitellogenin accumulation in developing oocytes and an inhibition of JH-regulated remodeling of follicular epithelium, suggesting a dopamine function of ovarian maturation with JH mediation (Bai and Palli, 2016). In *D. melanogaster*, sexually mature females express higher D1-like receptor (DopR) in the fat body than young immature females (Gruntenko et al., 2012). This expression pattern of the D1-like receptor in the fat body is similar to that in the ant *Diacamma* (Okada et al., 2015). Dopamine also regulates mating behavior in *D. melanogaster* (Wicker-Thomas and Hamann, 2008). Dopamine production regulated by JH has been reported in the brains of females in *D. melanogaster* (Argue et al., 2013) and *L. hesperus* (Brent et al., 2016). Thus, the reproductive function of dopamine is shared among solitary species across different orders and could be an ancestral character as a functional system to promote reproduction with JH.

DIVISION OF LABOR AMONG WORKERS

Workers change their tasks depending on their age, size, and/or morphology in the primitive and advanced eusocial hymenopterans. In contrast to the age-related division of labor which has been extensively studied in the honey bee, little is known about endocrine influences on the size-related division of labor that is seen commonly in ants and bumble bees. The size and morphology of workers performing different tasks are determined by developmental processes during the larval and pupal stages. Therefore, the size-related or morphological division of labor might be based on the particular physiological state of each subcaste after eclosion and can be modified by the states changing with age.

JH Function Regulating Age-Related Division of Labor

The physiological basis of the age-related division of labor has been studied extensively in honey bees. Younger bees perform tasks inside the nest, such as feeding larvae, constructing and maintaining the nest, and processing honey, whereas older bees guard the nest and forage (Winston, 1987). The onset of foraging in honey bees is linked to the action of JH (Robinson and Vargo, 1997; Bloch et al., 2009). JH titers in honey bee workers normally increase with age. Foraging worker bees have higher hemolymphatic JH titers than bees working in the nest. Treatment of young bees with methoprene, a JH analog, results in the initiation of foraging earlier in life. JH is produced in the corpora allata, paired secretory glands located close to the brain (Nijhout, 1994). Removal of the corpora allata resulted in bees that were able to initiate foraging although they were delayed in their foraging onset (Sullivan et al., 2000). These results not only demonstrate an effect of JH on age at onset of foraging but also show that JH is not necessary for the initiation and maintenance of foraging behavior. Therefore, it is likely that foraging onset in worker bees is governed by redundant control mechanisms. Such a JH function regulating the age-related division of labor has been reported in other advanced eusocial species such as the paper wasp *Polybia occidentalis* (O'Donnell and Jeanne, 1993) and some ants (*Diacamma*: Sommer et al., 1993; *Harpegnathos saltator*, Penick et al., 2011; **Figure 1**).

The JH function promoted foraging, including sugar response and learning has been reported in the solitary bee *Nomia melanderi* (Kapheim and Johnson, 2017) and the primitive eusocial paper wasp *Polistes canadensis* (Giray et al., 2005) and *Synoeca surinama* (Kelstrup et al., 2014b; **Figure 1**). Studies in the primitive eusocial species seem to support the SFH assuming the ancestral reproductive function of JH became split into a gonadotropin and a regulator of worker foraging in the primitive eusocial species. However, a study in *N. melanderi* did not support the SFH, because the hypothesis does not assume the ancestral foraging function by JH, rather this foraging function of JH supports the OGPH (Kapheim and Johnson, 2017). Therefore, the conclusion that the evolutionary process of the JH function shifted from a gonadotropin to a regulator of worker foraging is still controversial.

Social Behaviors and Physiology Mediated by Biogenic Amines

From Nursing to Foraging

Although nursing is a major task for workers in the nest, a clear involvement of biogenic amines in enhancing brood care has not been reported in hymenopterans, and increased levels of amines are more likely to be associated with extranidal tasks. An example of amine involvement in intranidal task regulation in honey bees could be tyramine and octopamine involvement in thermoregulatory fanning in the nest (Cook et al., 2017). The nursing behavior of worker honey bees occurs under lower levels of octopamine, dopamine, and serotonin, which increase in the brain with age under queenright conditions (Taylor et al., 1992; Schulz and Robinson, 1999). Interestingly, such age-related increases in these amines in the brains of workers are also observed in some ants, including *Pheidole* (Seid and Traniello, 2005), *Diacamma* (Okada et al., 2015), and the jumping ant *Harpegnathos* (Penick et al., 2014). For example, in *Pheidole dentata*, serotonin modulates minor worker responsiveness to trail pheromones (Muscedere et al., 2012). These facts suggest that the age-dependent increase in octopamine, dopamine, and serotonin is a shared pattern in worker behavioral ontogeny, at least in the honey bee and ants.

Octopamine in the brain is enhanced by JH and promotes foraging behaviors in the honey bee (Schulz and Robinson, 2001; Schulz et al., 2002). Octopamine can enhance the responses of nestmate recognition with aggression and learning in workers (Robinson et al., 1999; Farooqui, 2012), which could support guarding behaviors initially and then foraging behaviors that require learning and memory. Octopamine also mediates the persistent modulation of associative learning and memory induced by an attractive pheromone component geraniol (Baracchi et al., 2020). Expression of a gene encoding the octopamine receptor (*Amoct*β*r3/4*) changes with age rather than with social task, whereas that of *Amoct*α*r1* correlates with social tasks (Reim and Scheiner, 2014). Octopamine can also enhance sugar responses in the proboscis extension of the honey bee (Scheiner et al., 2002) and the stingless bee *Melipona scutellaris* (McCabe et al., 2017), which is a behavioral modulation for foraging characteristics. In fact, octopamine increases individual foraging effort and collective food source exploitation in the neotropical stingless bee *Plebeia droryana* (Peng et al., 2020). In terms of foraging behaviors, brain levels of octopamine are higher in dancing honey bees than in those that follow them, and octopamine selectively increases the reporting of resource value in dances by foragers (Barron et al., 2007).

Aggression for Guarding or Predation

The involvement of several biogenic amines in defensive or aggressive behaviors has been reported in social hymenopterans. Aggressive behaviors can be categorized into interactions within species (intraspecies) and between species (interspecies). The effects of amines in aggressive dominance interactions are discussed earlier (see section "Social Factors Affecting Dopamine Levels in the Brain"). Interspecies aggression can be further divided into attacks during foraging (predation), nest defense against predators, and competition between species. Octopamine is known to elevate aggression levels between conspecific males generally in solitary insects, including fruit flies (Baier et al., 2002) and crickets (Stevenson et al., 2005). These interactions between rival males might correspond to intraspecies interactions in social species. In honey bee workers, several octopamine agonists enhance the aggression levels during nestmate recognition (Robinson et al., 1999). Serotonin enhances the response to the alarm pheromone component (Harris and Woodring, 1999; Nouvian et al., 2018). In ants, octopamine and serotonin might be involved in aggressiveness in intra- and interspecies interactions. Although the functional differences between octopamine and serotonin are unknown, there are positive correlations between aggressiveness and octopamine in intraspecies interactions in *Oecophylla smaragdina* (Kamhi et al., 2015), and in interspecies interactions in *F. japonica* (Aonuma and Watanabe, 2012b) and *O. smaragdina* (Kamhi et al., 2015), or between aggressiveness and serotonin in intraspecific interactions in *Formica rufa* (Kostowski and Tarchalska, 1972; Kostowski and Tarchalska-Krynska, 1975). In *Odontomachus kuroiwae*, oral administration of serotonin or its precursor strongly promotes the initiation of defensive behavior (Aonuma, 2020). In general, worker aggressiveness increases with age in social Hymenoptera. Together with the results discussed in the previous section, the age-dependent increase in biogenic amines might generally enhance the aggressiveness of workers, although there could also be context-dependent adjustments of aggression levels in these workers.

Cooperative behaviors in social insects can be observed under conditions of low aggressiveness among nestmates. In the ant *Camponotus fellah*, octopamine decreases the frequency of trophallaxis (food exchange) between nestmate workers, whereas the application of serotonin does not affect trophallactic frequencies, suggesting that octopamine has a stronger effect on trophallaxis than does serotonin (Boulay et al., 2000). In the ant *Pristomyrmex punctatus*, the intake of secretion of the myrmecophile butterfly *Narathura japonica* larvae causes a low level of brain dopamine that enhances the intensity of guarding behavior of workers to the latter (Hojo et al., 2015).

DISCUSSION

The OGPH assumes the decoupling of reproductive physiology into reproductive and non-reproductive states that are allocated to different individuals in eusocial species (West-Eberhard, 1996). Given that biogenic amines have multiple roles in reproduction and general behavior, we expect that the involvement of biogenic amines in the regulation of physiological status between reproductives and non-reproductives is derived from the characteristics of the solitary ancestors of eusocial species (Kamhi et al., 2017; **Figure 4**). The reproductive physiology in primitive and advanced eusocial hymenopterans is broadly associated with dopamine in the brain (**Figure 1**, see section "Reproductive Division of Labor and Worker Reproduction"), whereas non-reproductive behaviors in advanced eusocial species, including foraging and nest defense,

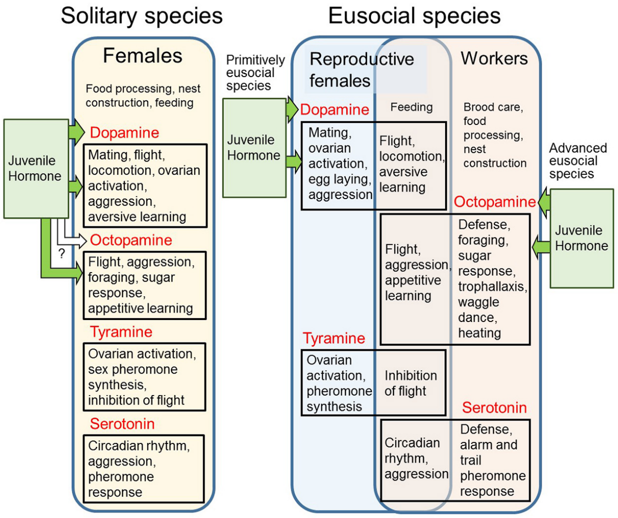


FIGURE 4 | Decoupling the multiple roles of biogenic amines into each phenotype in eusocial species. Multiple roles of biogenic amines in solitary and eusocial species are presented. Several general behaviors or movements are shared between both reproductive females and workers in eusocial species (overlapping area). Juvenile hormone regulates dopamine in solitary and primitively eusocial species, whereas it regulates octopamine in advanced eusocial species.

are mainly mediated by octopamine and serotonin (Farooqui, 2012; see section "Division of Labor Among Workers"). Equivalent roles of dopamine in reproduction (mating and ovarian activation) (see section "Reproductive Function of Dopamine in Solitary Insects") and octopamine in foraging (sugar response, appetitive learning, and memory) (Roeder, 2005; Farooqui, 2012) are found in a single individual in solitary species. Given such preadaptation, the OGPH would be supported (**Figure 4**). There are, however, several general behaviors or movements that are shared between both reproductives and non-reproductives in eusocial insects. For example, flight behavior is necessary not only for foraging in non-reproductives but also for nuptial flight in reproductives. Given that octopamine and dopamine are tightly related with flight (reviewed by Roeder, 2005; Farooqui, 2012, see sections "Reproductive Division of Labor and Worker Reproduction" and "Division of Labor Among Workers"), the roles of these monoamines might be shared between both reproductives and non-reproductives. Thus, specialization of behavior might require and/or renounce particular sets of aminergic function originated from a pool of ancestral aminergic function.

Generally, in adult insects, JH is involved in physiology and behaviors for both reproductive and non-reproductive states in solitary species (Hartfelder, 2000; Raikhel et al., 2005; Kapheim and Johnson, 2017; Trumbo, 2018). JH is a primary regulator of reproduction, including ovarian activity and mating behaviors (Hartfelder, 2000; Raikhel et al., 2005) with dopamine function, and is also involved in foraging, including sugar response and learning (Kapheim and Johnson, 2017; Trumbo, 2018; **Figure 4**). In eusocial hymenopterans, the reproductive function of JH is conserved in primitive eusocial species, whereas JH promotes age-polyethism in non-reproductives in advanced eusocial hymenopteran species (**Figure 1**). In advanced eusocial species, loss of JH reproductive function leads to the SFH, whereas the evidence contradicts the OGPH because the JH reproductive function is not conserved in reproductive individuals. In contrast, the evidence of JH foraging function in solitary species does not support SFH, rather it fits the situation for OGPH. Interestingly, the reproductive function of dopamine and foraging function of octopamine are widely conserved in solitary to advanced eusocial species. The function expressed in a single individual in solitary species was decoupled into queens and workers in eusocial species, supporting the OGPH. Corresponding to this function, JH regulates dopamine in reproductive individuals in primitive eusocial species (see section "Social Factors Affecting Dopamine Levels in the Brain"), whereas it is positioned upstream of octopamine in non-reproductives in advanced eusocial species (see section "Social Behaviors and Physiology Mediated by Biogenic Amines") (**Figure 4**). This rewiring of the signal transduction networks between JH and biogenic amines can explain the mechanism underlying the shift of JH function from a gonadotropin to a regulator of worker foraging and might make it possible to regulate reproduction in response to various social stimuli.

PERSPECTIVE

Biogenic amines are broadly present in the nervous systems of invertebrates, modulating their behaviors and reproduction. A limited number of substances can regulate diverse behaviors in eusocial hymenopterans by local secretion in the CNS, or by combining the effects of several monoamines, or their dose-dependent effects. Reports on the behavioral effects of biogenic amines are increasing, although the regulatory systems of biogenic amines, including their interactions with hemolymph hormones or signaling molecules, remain unclear. Important topics for future research include: (i) more functional validation of the biogenic amines associated with social behaviors; (ii) clarification of the "crosstalk" with other physiological mechanisms in signal transduction and regulatory networks; and (iii) widely comparative studies of females in solitary, facultative, and primitive eusocial species as a model of the ancestral mode of reproductive physiology. Taxa-wide comparative studies incorporating phylogenetic information must be efficacious so that the preadaptation of physiological mechanisms and its roles in social evolution can be understood. Comparative studies would also be necessary to reveal both the generality and specificity of physiological mechanisms for social characters across taxa.

CONCLUSION

The OGPH assumes the physiological decoupling of ovarian cycles and behavior into reproductive and non-reproductive states. The multiple roles of biogenic amines in social behavior and reproduction provide evidence for this hypothesis. Dopaminergic signaling has a pivotal role in the formation of reproductive skew irrespective of the social system, whereas octopaminergic signaling contributes largely to non-reproductive social behaviors. These roles of biogenic amines occur in the neuroendocrine system throughout the life cycle of solitary species, supporting the OGPH. JH promotes reproduction with dopamine in primitive eusocial species, whereas it regulates non-reproductive social behaviors with octopamine in advanced eusocial species. Thus, the signal transduction networks between JH and biogenic amines have been rewired in advanced eusocial species, which makes it possible for these species to regulate their reproduction in response to various social and environmental stimuli.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. KS wrote the majority of the manuscript and other authors edited the manuscript on their expertise.

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When and Why Did Human Brains Decrease in Size? A New Change-Point Analysis and Insights From Brain Evolution in Ants

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Human brain size nearly quadrupled in the six million years since *Homo* last shared a common ancestor with chimpanzees, but human brains are thought to have decreased in volume since the end of the last Ice Age. The timing and reason for this decrease is enigmatic. Here we use change-point analysis to estimate the timing of changes in the rate of hominin brain evolution. We find that hominin brains experienced positive rate changes at 2.1 and 1.5 million years ago, coincident with the early evolution of *Homo* and technological innovations evident in the archeological record. But we also find that human brain size reduction was surprisingly recent, occurring in the last 3,000 years. Our dating does not support hypotheses concerning brain size reduction as a by-product of body size reduction, a result of a shift to an agricultural diet, or a consequence of self-domestication. We suggest our analysis supports the hypothesis that the recent decrease in brain size may instead result from the externalization of knowledge and advantages of group-level decision-making due in part to the advent of social systems of distributed cognition and the storage and sharing of information. Humans live in social groups in which multiple brains contribute to the emergence of collective intelligence. Although difficult to study in the deep history of *Homo*, the impacts of group size, social organization, collective intelligence and other potential selective forces on brain evolution can be elucidated using ants as models. The remarkable ecological diversity of ants and their species richness encompasses forms convergent in aspects of human sociality, including large group size, agrarian life histories, division of labor, and collective cognition. Ants provide a wide range of social systems to generate and test hypotheses concerning brain size enlargement or reduction and aid in interpreting patterns of brain evolution identified in humans. Although humans and ants represent very different routes in social and cognitive evolution, the insights ants offer can broadly inform us of the selective forces that influence brain size.

Keywords: brain, evolution, ant, hominin, Holocene

> *We live in a community of knowledge. Everything we do depends on knowledge that is both inside our head as well as out in the world and in other people's heads.*

Steven Slomen[1], author of *Knowledge Illusion: Why We Never Think Alone*

> *The key to the origin of the human condition is not to be found in our species exclusively, because the story did not start and end with humanity.*

E. O. Wilson, *The Social Conquest of Earth*

> *Only humans and social insects can build and manage large-scale societies according to complex economic decision rules.*

Boomsma and Franks, 2006

INTRODUCTION

Understanding the causes and consequences of brain evolution in humans—particularly the role of social life—is significant to understanding the nature of humanity. Across diverse clades, sociality is hypothesized to drive brain size and structure. In primates, greater cognitive challenges associated with forming bonded social groups in large societies, among other influences (DeCasien et al., 2017; González-Forero and Gardner, 2018; DeCasien and Higham, 2019), appear to have selected for increased brain size (Dunbar, 1998; Dunbar and Shultz, 2007, 2017; Meguerditchian et al., 2021). A broad phylogenetic perspective can be of significant value in exploring the evolution of nervous systems (Striedter et al., 2014; Keifer and Summers, 2016; Shigeno, 2017). Although significantly different in sociality, computation and decision-making in humans and social insects are accomplished by physical neuroarchitectures ("solid brains") as well as "liquid brains" formed by interactions of group members that create collective intelligence (Couzin, 2009; Pagán, 2019; Piñero and Solé, 2019; Reséndiz-Benhumea et al., 2021). Across diverse species that vary in social organization, cognitive demands on individuals may be lower in societies in which group decision-making is more efficacious than individual decision-making (Surowiecki, 2004; Sumpter, 2006; Krause et al., 2010; Woolley et al., 2010; Sasaki and Pratt, 2018; Bak-Coleman et al., 2021). Superorganismic decentralized "brain" networks characterize humans and ants, the premier social insect. Collective intelligence may reduce brain size in both clades (Bailey and Geary, 2009; Feinerman and Traniello, 2016). Therefore, the size of groups and society-level intelligence may affect behavioral performance and cognitive loads and increase or reduce brain size, depending on context.

Over the course of hominin evolution, encephalization has been dynamic (e.g., Miller et al., 2019). *Australopithecus* cranial capacities were on average 20% larger than those of the late Miocene hominins *Sahelanthropus* and *Ardipithecus* or modern chimpanzees, despite having chimpanzee-sized bodies (Wolpoff, 1999; Cartmill and Smith, 2009; DeSilva, 2011). These ∼450 cc brains remained roughly unchanged in size from 3.5 to 2.0 million years ago even though late australopiths (e.g., *Paranthropus*) underwent extensive diversification. With the evolution of *Homo*, brains began to expand but gross neural organization may have remained primitive (Ponce de León et al., 2021). Additionally, brain expansion was not universal in fossil *Homo* as evidenced by the small-brained Middle and Late Pleistocene hominins *Homo naledi* (Berger et al., 2015; Montgomery, 2018) and *Homo floresiensis* (Brown et al., 2004; **Figure 1**). Although an almost fourfold increase in brain volume during the last 2 million years is a hallmark in human evolution, it remains unappreciated—but well-documented—that both absolute and relative brain size have *decreased* since the end of the Pleistocene (Schwidetzky, 1976; Wiercinski, 1979; Beals et al., 1984; Henneberg, 1988; Henneberg and Steyn, 1993; Ruff et al., 1997; Bailey and Geary, 2009; Hawks, 2011; Bednarik, 2014; Liu et al., 2014; Bruner and Gleeson, 2019). The precise timing of this decrease in brain size, however, is unclear. Some have placed its origin in the late Pleistocene ∼35 kyr (Ruff et al., 1997) and others in the more recent Holocene ∼10 kyr (Henneberg, 1988; Hawks, 2011).

Here we investigate historical patterns of human brain evolution to date major inflection points of changes in size to attempt to identify selective factors in the environment that may have prevailed during times of significant change in brain size. Because the deep history of neural tissue and its organization is difficult to explore, we use ants to model the broad impacts of social selection on brain size evident in nature to gain insights into the possible selective forces that influenced patterns of human brain evolution. Although phylogenetically remote from humans, ants provide examples of brain evolution that may help identify selective factors and offer neuroarchitectural details to complement the metric of brain size. These advantages may compensate for some of the limitations of behavioral and neurobiological research on extinct hominin forms.

Ants are eusocial insects characterized by reproductive division of labor, cooperative brood care, and overlap of generations. They are exemplars of social life that encompass extant species basal in social structure and highly complex species, enabling comparative studies. Ants are eusocial, and humans have been characterized as eusocial (Foster and Ratnieks, 2005; Boomsma and Franks, 2006; Betzig, 2014; D'Ettorre, 2017b), both forming large, complex, kin-oriented societies, including those with agricultural practices and full-time division of labor. In humans, however, division of labor is not associated with a loss of reproductive potential. *Ultrasocial* species (Campbell, 1983; Gowdy and Krall, 2013, 2016) produce their own food crops and include some ants (and termites) and humans. Sociobiological parallels can be leveraged to understand the general role of advanced social life in brain evolution. While divergent in key aspects of social organization, humans and ants exhibit important convergences, for example, in the ability to act collectively. And although their brains are structurally and functionally different, our understanding of brain size scaling and structural allometries in ants provide opportunities to address general questions of brain evolution—including size reduction — in humans.

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[1] From *Big Picture Science* podcast, Collective "Knowledge." May 17, 2021.

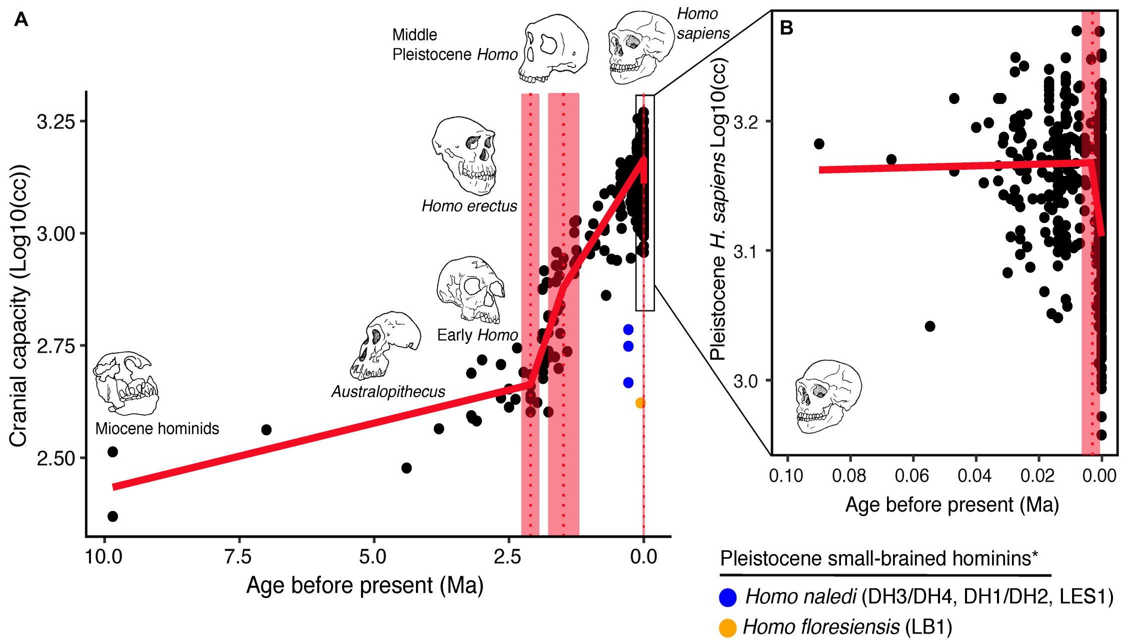

FIGURE 1 | Trends in hominin brain evolution. **(A)** Cranial capacity in fossil apes (Miocene hominids) and hominins over the last 10 million years. Brain size remained relatively steady throughout the late Miocene and Pliocene, increasing only slightly in *Australopithecus* compared with earlier hominins. However, by 2 million years ago, there was a dramatic increase in the rate of growth, coinciding with fossil evidence for the earliest members of genus *Homo*. This change point is illustrated by a red vertical dotted line (95% CI shown as thick pink vertical line). A second change-point is detected at ~1.5 million years ago and the rate of brain size increase remains steady through the Pleistocene and the evolution of *Homo sapiens*. If the small-brained Middle and Late Pleistocene hominins *H. naledi* (blue dots) and *H. floresiensis* (orange dot) are included, these two change points merge into a single, overlapping encephalization event between 1.97 and 2.21 million years ago. **(B)** During the last 100,000 years, brain size has remained steady in *H. sapiens* until a rapid and dramatic change point only 3,000 years ago decreased Holocene human brain size at a rate fifty times greater than the previous increases in Pleistocene brain volume. Each black dot represents an individual fossil skull or osteological specimen.

MATERIALS AND METHODS

To understand temporal patterns of human brain evolution, we applied a change-point analysis to identify the timing of inflection points in hominin brain evolution using our brain size dataset of 985 dated $\log_{10}$ transformed estimates of hominin cranial capacities (cc) compiled from the literature (**Supplementary File 1**). The dataset represents brain evolution over the last 10 million years of hominid and hominin evolution and includes *Rudapithecus* ($N = 2$), *Sahelanthropus* ($N = 1$), *Ardipithecus* ($N = 1$), *Australopithecus* (including *Paranthropus*) ($N = 29$), Early Pleistocene *Homo* ($N = 37$), Middle Pleistocene *Homo* ($N = 60$), Late Pleistocene *Homo* ($N = 156$), and Holocene *H. sapiens* ($N = 699$). We only used published cranial volumes (cc or mL) and not cadaver-derived weights (g) since cranial volume and brain weight are not equivalent (e.g., Tobias, 1970). Analysis was limited to individuals that are estimated to have been at least 10 years old and thus had exceeded the age at which modern *H. sapiens* achieves adult brain volume (Coqueugniot and Hublin, 2012).

Changepoints were determined using the packages *changepoint* (Killick and Eckley, 2014) and *segmented* (Muggeo, 2008) in R Studio (Version 1.2. 5019). Changepoint was first used to provide approximate prior estimates for changes in the mean $\log_{10}$(cc) across the time series using the "BinSeg" method. These prior estimates were then used to fit a piecewise generalized linear model to the data with the *segmented* package (Muggeo, 2008), which provided estimates of 1) the locations of changepoints (or breakpoints) in the slope of the time series; and 2) the slopes of the lines around each changepoint, which we interpreted as approximate rates of evolutionary change. Because the phylogenetic relationship of the different hominin species remains contentious and because there is genetic evidence for interbreeding between many Late Pleistocene hominin populations (e.g., Gokcumen, 2020), we included all hominin specimens in the change point analysis. However, we ran two separate models: one including the small-brained Middle and Late Pleistocene fossils from *H. naledi* and *H. floresiensis* and one excluding them. We mainly describe the results for when these small brained species were excluded ($N = 981$), but the timing of the decrease in hominin brain size in the Holocene was negligible between these two models.

RESULTS

The best piecewise GLM model fit explained approximately 79% of the variance (adj $r^2 = 0.79$) in $\log_{10}$(cc) values and identified three changepoints (**Table 1**). The first was detected at 2.10 ± 0.07 Ma, coincident with fossil evidence near the

TABLE 1 | Estimates of changepoint dates—excluding small-brained Pleistocene *Homo* (N = 981).

Changepoint (CPT)	Date (Ma) ± SE	95% Confidence Interval (CI) for changepoint date (Ma)	Rate of change after changepoint [(log10(cc)/Ma] ± SE
CPT 1	2.10 ± 0.07	2.25 < CPT 1 < 1.96	0.35 ± 0.05
CPT 2	1.49 ± 0.14	1.75 < CPT 2 < 1.22	0.19 ± 0.01
CPT 3	0.003 ± 0.001	0.005 < CPT 3 < 0.001	−17.16 ± 6.69

TABLE 2 | Estimates of changepoint dates—including small-brained Pleistocene *Homo* (N = 985).

Changepoint (CPT)	Date (Ma) ± SE	95% Confidence Interval (CI) for changepoint date (Ma)	Rate of change after changepoint [(log10(cc)/Ma] ± SE
CPT 1	2.10 ± 0.05	2.19 < CPT 1 < 2.01	0.72 ± 0.51
CPT 2	1.97 ± 0.12	2.21 < CPT 2 < 1.73	0.20 ± 0.01
CPT 3	0.003 ± 0.001	0.006 < CPT 3 < 0.0005	−16.74 ± 7.55

first known occurrence of *Homo erectus* (Herries et al., 2020). At 2.10 Ma, the rate of evolution increased sharply from 0.03 ± 0.01 $\log_{10}$(cc)/Ma to 0.35 ± 0.05 $\log_{10}$(cc)/Ma. A second changepoint occurred at 1.49 ± 0.14 Ma when the rate of evolution slowed to 0.19 ± 0.01 $\log_{10}$ (cc)/Ma. A steady increase in brain size—independent of body size—followed and lasted through the Pleistocene (Ruff et al., 1997; Lee and Wolpoff, 2003; Rightmire, 2004; Hawks, 2011). We identified a third changepoint at 0.003 ± 0.001 Ma at a rate of -17.16 ± 6.69 $\log_{10}$(cc)/Ma. This rate is 50 times greater than the renowned increase in human brain size. Our data suggest that this reduction may have been more recent—3,000 years ago—than previously suggested. We interpret our result conservatively, and caution that any findings about brain size changes throughout human evolution are contingent on the resolution of the available dataset (e.g., VanSickle et al., 2020). Indeed, the inclusion of smaller-brained Pleistocene hominins (*H. naledi* and *H. floresiensis*) reduced model fit slightly (adj $r^2 = 0.74$) and had the effect of widening the 95% CIs of the first two changepoints such that they overlapped between the wider time interval of 2.2–1.7 Ma (**Table 2**). Yet the timing of the third Holocene changepoint in this model was unaffected by the inclusion of these specimens, although its 95%CI also widened slightly (**Table 2**).

DISCUSSION

Brain Size Increase and Reduction in Humans

The changepoints we identified document trends in brain size but do not reveal underlying causes and mechanisms of encephalization. The expensive tissue hypothesis (Aiello and Wheeler, 1995) posits that a trade-off in allocated resources from one expensive tissue (the brain) to another (the gut) was made possible by shifts to a higher quality diet in *Homo*, enabled in part by enhanced technological skills (Lepre et al., 2011), exploitation of diverse resources (Braun et al., 2010), and the invention of cooked foods through controlled fire (Wrangham, 2009; Herculano-Houzel, 2016). In addition to evidence that energetic constraints on encephalization may have been released by dietary shifts in early *Homo*, others have posited that brain expansion in early *Homo* may have been driven by the need for enhanced social intelligence (Dunbar, 1998; Dunbar and Shultz, 2017). With growing complexity in social life, perhaps associated with increased group size, brain expansion occurred. Resource sharing within groups and allocare may have provided the energy surplus needed to support the increased energetic cost of a larger brain (Isler and van Schaik, 2012). These hypotheses are not mutually exclusive: a combination of social challenges and ecological pressures and increased dietary quality and breadth drove Pleistocene brain expansion in our ancestors, who were living in increasingly larger groups and likely benefitted from enhanced group-level cognitive abilities and greater cultural intelligence (van Schaik et al., 2012), which could in turn accelerate brain expansion via a feedback loop (Markov and Markov, 2020).

The cause of brain reduction in the Holocene is also unclear. One possibility is that it is associated with a corresponding decrease in body mass (Henneberg, 1988; Ruff et al., 1997). The early expansion of the brain in *Homo* has been explained as an increase in absolute body size (Ruff et al., 1997; Wood and Collard, 1999; McHenry and Coffing, 2000; Lee and Wolpoff, 2003) though Grabowski (2016) later employed quantitative genetics to suggest that selection had favored brain enlargement and body size increase was a by-product. Hawks (2011) contends that the absolute change in brain size from the Pleistocene to the Holocene is greater than expected based on changes in body mass in the same time period, emphasizing that given the correlation between human brain and body size (Holloway, 1980), the observed 5 kg decrease in body size in the Holocene would account for only a 22 mL decrease in brain volume (Hawks, 2011). However, the actual reduction is more than 5x greater, suggesting that body size alone cannot entirely explain the decrease in brain volume. We find here that body size reduction may have preceded brain size reduction by several millennia. Ruff et al. (1997) notes that the decrease in body size in late Pleistocene humans began around 50 kyr, whereas brain reduction appears to been a Holocene phenomenon.

Yet there may be other non-allometric explanations for the reduction in human brain size at the Pleistocene-Holocene transition due to energetic, nutritional (Wiercinski, 1979), and/or developmental constraints (Hawks, 2011). The brain size reduction in Holocene humans parallels that of domesticated animals, suggesting that humans have self-domesticated by deliberately removing highly aggressive individuals from breeding populations, leading to a reduction in intra-population (but not inter-population) aggression (Leach, 2003; Hare and Tomasello, 2005; Wrangham, 2018, 2019; Bruner and Gleeson, 2019; Hare and Woods, 2020). Brain reduction in this case would be a by-product (or cause) of docility, a phenomenon documented recently in domesticated cattle (Balcarcel et al., 2021). Groves (1999) suggests that domesticated dogs have sufficiently co-evolved with humans to have symbiotically become our external senses, thereby decreasing our reliance on brain centers that process sensory information. However, our finding here—that brain size reduction occurred in just within last 3,000 years—is temporally inconsistent with these prior explanations. Human self-domestication is argued to have occurred at the onset of species ∼300,000 years ago (Wrangham, 2019) or coincident with the evolution of what some have called "behavioral modernity" ∼80,000 years ago (Hare and Woods, 2020). Recent fossil and genetic evidence indicate that dogs were domesticated > 20,000 years ago (Perri et al., 2021).

Human and Ant Sociobiology and Evolutionary Neurobiology: Insights Into Brain Elaboration and Reduction

Human social life has long been analyzed in reference to the social organization of ants. Imms (1946) review of Haskins (1939) *Of Ants and Men* described ants as "predominant" among the "very few living creatures whose social development at all parallels our own." He emphasized the value of having "a nearly complete series of evolutionary forms among living ants, numerous 'missing links' and 'living fossils'" and a history "much more complete and much better preserved than that of man." Comparisons made to identify commonalities in patterns of sociality between these diverse clades continue to be made today (Boomsma and Franks, 2006; Wilson, 2012; Crespi, 2014; D'Ettorre, 2017a,b; Friedman and Søvik, 2021; Gowdy, 2021). The benefits of ant models for the study of human social evolution noted by Imms are made more compelling by an additional 80 + years of new species discovery (roughly 5,000–6,000 circa 1,940 to > 14,000 today), an expanded phylogeny (6 to ∼20 subfamilies), and an eruption of integrative sociobiological and neurobiological research. Ants exhibit striking variation in colony size and demography, and individual and colony-level cognition. Diversity in social phenotypes creates the potential to provide general insights into human social structure and brain evolution.

Acknowledging the limitations of analogies, human and ant comparisons may reveal patterns in nature that broadly suggest social and ecological selective forces relevant to brain evolution. We recognize that the uniqueness of human cognition (Laland and Seed, 2021) cannot be overemphasized, and neurobiological parallels with ants are as constrained as sociobiological comparisons, if not more limited. Although ant and human brains have the common function of processing environmental information to adaptively respond to social signals and cues, they are structured very differently. The size, neuron number, and synaptic connectivity of an ant brain is a minute fraction of that of a human brain (Herculano-Houzel, 2016; Godfrey et al., 2021). However, the computational power of an ant brain is remarkable for its size and miniaturization does not appear to constrain behavioral performance and/or higher-order processing (Muscedere et al., 2014), social learning, or consciousness (Avarguès-Weber and Giurfa, 2013; Barron and Klein, 2016; Perry et al., 2017; Lihoreau et al., 2019; Perry and Chittka, 2019; Elek et al., 2020; D'Ettorre et al., 2021). Social organization in ants may "require relatively simple and computationally inexpensive forms of cognition" (Lihoreau et al., 2012), some extrinsic to brain operations, that could mitigate the need for advanced processing capability. Behavior in ants typically involves task routines and kinesthetic performance rather than sophisticated cognition. Unlike the human brain (Noonan et al., 2018), ant brains appear to lack executive function and the ability to mentalize, among other circuitry specialized for human social performance.

Relative needs for social information processing are strikingly different in ants and humans. Olfaction is dominant in ants and involves a relatively small number of chemicals to guide individual actions and organize colony-level behavior. Vision appears to have very limited social functions and mainly functions in navigation during foraging (Hölldobler and Wilson, 1990). The anatomy of ant brains thus largely reflects investment in brain centers such as the antennal lobes and optic lobes responsible for processing olfactory and visual stimuli, respectively. These inputs are mainly integrated in the mushroom bodies, brain compartments specialized in higher-order processing, learning, and memory, and to a lesser extent in the central complex. The mushroom bodies, deeply homologous to the vertebrate cortex (Tomer et al., 2010), have long been consider to be the neuroanatomical seat of intelligence in ants and other insects (Strausfeld et al., 1998), but the nature of ant intelligence is only very loosely comparable to that of humans. Mushroom body elaboration also preceded the evolution of eusociality (Farris and Schulmeister, 2011).

Ants have nevertheless emerged as important models for understanding the role of sociality (Ilies et al., 2015; Kamhi et al., 2016, 2019; Godfrey and Gronenberg, 2019) and behavioral performance and cognition (Muratore and Traniello, 2020; Muratore et al., 2021) in brain evolution. Analyses of sociality and brain size scaling (e.g., Godfrey and Gronenberg, 2019; Muratore and Traniello, 2020) and information processing through social interaction (e.g., Davidson et al., 2016) can contribute to broadly understanding how social biology may have influenced general aspects of human brain evolution. The allometric scaling of functionally specialized brain centers in ants allows the adaptiveness of brain mosaics to be explored (e.g., Muratore et al., 2021) and the metabolic costs of neural tissues that are highly significant to brain evolution (Aiello and Wheeler, 1995) can be recorded in individual ant brains (Coto and Traniello, 2021). Studies of the influences of diet, which is considered significant in primate (and human) brain evolution (Wrangham, 2009; DeCasien et al., 2017), are facilitated by the diverse feeding ecologies of ants. Ant nutritional socioecology spans highly specialized as well as generalist predators that vary in colony size and degree of complexity of social organization, allowing the relative roles of diet and social complexity to be separated because specialist and generalist species that each form small and large colonies (Azorsa et al., in preparation). Ants also have culture in the form of tool use (Zhou et al., 2020 and references therein), and although they do not process food by cooking, they may preserve prey (Maschwitz et al., 1979).

The application of theories of vertebrate brain evolution to social insects has been debated (Lihoreau et al., 2012; Farris, 2016) and studies describe variable relationships between sociality, brain and brain compartment size, body size, and group size in eusocial insects (Farris and Schulmeister, 2011; Seid et al., 2011; Riveros et al., 2012). Workers of ant species characterized by larger colony size have larger brains, suggesting greater social interaction selects for increased brain size (Wehner et al., 2007). Similarly, comparisons of socially basic and social complex ant species suggest larger colony size and collective actions have selected for larger brains and mushroom bodies (Kamhi et al., 2016). In contrast, in monomorphic fungus-growing ants, which have agrarian habits, larger colony size is associated with decreased brain size (Riveros et al., 2012). This latter comparison among agricultural ant species provides insight into how increased group size and sociality in human populations may have reduced brain size (Bailey and Geary, 2009) due to a high level of emergent complexity.

Worker behavior undergoes age-related development in ants. Like human brains, ant worker brains exhibit age-related synaptic remodeling, suggesting synaptic pruning in association with an increasingly diverse, flexible, and efficient behavioral repertoire (Seid et al., 2005; Seid and Traniello, 2006; Muscedere et al., 2009). Age and behavioral development can be associated with increased brain volume (Muscedere and Traniello, 2012) and declines in the density of mushroom body microglomeruli (Gordon and Traniello, 2018)—"microprocessor"-like synaptic structures that underlie plasticity in sensory processing capability and behavior (Groh and Rössler, 2011; Groh et al., 2014). Elements of human brain development are thus mirrored in ant brain ontogeny and social behavior. In mole rats, eusocial mammals convergent in social structure with eusocial insects, there is no clear association between social system and relative brain size: eusocial species do not have smaller or larger brains than social or solitary species (Kverková et al., 2018). Although there is an apparent trend between social system and forebrain neuron number, this is likely an artifact of the extreme metabolic adaptations to subterranean life found in *H. glaber* (Kverková et al., 2018; Browe et al., 2020). Ants appear to provide more useful models than eusocial mammals.

Division of Labor and Brain Investment Patterns

Division of labor is a core social trait in humans and ants that potentially influences brain size evolution by unequally distributing behavioral performance needs and cognitive loads across group members. Greater social complexity in ants is correlated with large group size and division of labor among polyphenic workers (Anderson and McShea, 2001; Kappeler, 2019). Division of labor by morphologically differentiated and behaviorally specialized workers (physical castes) is an attribute of a relatively small number of ant species. Analyses of brain and behavior in these ants, especially those featuring extraordinary polymorphisms and high degrees of behavioral specialization, can provide important information on how distributed cognition impacts the size and scaling of brain size and structure in relation to body size. For example, mushroom bodies are disproportionally large in the brains of media (mid-size) workers of the fungus-growing ant *Atta cephalotes*. The diverse and behaviorally challenging task repertoire of these workers encompasses leaf-harvesting, and their social role and neuroanatomy differs from that of smaller and larger workers that perform other tasks (Muratore et al., 2021). Using this ant as a model to understand how agriculture has influenced brain evolution may help identify social conditions, life histories, and ecological factors favoring either an increase or decrease in brain size. It can also help identify how brain centers responsible for higher-order processing have evolved in response to either narrowly circumscribed or pluripotent and flexible task repertoires. In humans, such changes may have occurred during the transition from hunter-gatherer to agrarian habits. Ants provide a window through which such evolutionary processes may have occurred, and neuroanatomical outcomes.

Other strongly polymorphic ants offer additional insights into the role of division of labor in the evolution of brain size and compartmental scaling (Muscedere and Traniello, 2012; Ilies et al., 2015; Gordon et al., 2017, 2019). Ecologically different species may have distinct neural phenotypes that vary in the size of sensory input compartments and the mushroom bodies, among other brain centers (Muscedere and Traniello, 2012). Mushroom body size correlates positively with task plasticity, supporting the notion that greater demands on behavioral performance are reflected in greater investment in higher-order processing tissues. Task specialization may also affect brain size and compartmental scaling: larger colonies may show a higher level of task specialization among workers, reflected in relatively larger mushroom bodies (Amador-Vargas et al., 2015). The larger picture is that brain differentiation can occur in association with division of labor in the absence of striking changes in body size, illustrating how selection may have operated in humans as social groups increased in size.

Collective Intelligence and Neuroenergetic Costs

Ponce de León et al. (2008) and Hawks (2011) suggested that reduction of human brain size—without any evidence for intellectual diminishment—implies selection for brain efficiency. In ants, there is evidence of selection for metabolic efficiency in the brain. Kamhi et al. (2016) used cytochrome oxidase (COX) activity, a proxy for neuron metabolism, to contrast brain evolution and social evolution in the weaver ant *Oecophylla smaragdina* and the garden ant *Formica subsericea*, two sister clades whose workers are equivalent in body size but differ strongly in social organization and collective intelligence. Increased social complexity in weaver ants—reflected in division of labor by worker physical castes, large colony size, and remarkable decentralized group action including cooperative nest building—was associated with larger mushroom bodies, implying greater needs for higher-order information processing.

Weaver ant worker mushroom bodies have reduced COX activity. Therefore, increased brain size in socially complex species may be associated with reduced brain operation costs, contrasting with the assumption that increased brain size increases metabolic costs (Aiello and Wheeler, 1995; Isler and van Schaik, 2009).

Social Selection and Brain Evolution

Reduction in brain size may not compromise cognitive performance if intelligence is an attribute of the society rather than the individual. Galton (1907) first described that the accuracy of decision making by human groups could exceed that of any individual group member. This concept of collective intelligence has since been elaborated in studies ranging from insects to humans (Woolley et al., 2010; Ward et al., 2011; Sasaki and Pratt, 2018; Almaatouq et al., 2020). If brain production, maintenance, and operation costs are metabolically significant (Aiello and Wheeler, 1995; Leonard et al., 2003; Isler and van Shaik, 2006; Chittka and Niven, 2009; Navarrete et al., 2011; Kuzawa et al., 2014; Pontzer et al., 2016; Herculano-Houzel, 2017), then collective intelligence may reduce demands for neural tissue to support individual cognitive capabilities. Using a multidisciplinary modeling approach, Reséndiz-Benhumea et al. (2021) indeed demonstrated that agents with relatively small brains can through social interaction achieve a level of behavioral performance comparable to those of larger-brained but solitary agents. Further testing of this idea, however, will require a better understanding of whether Holocene human brains reduced isometrically or if specific regions reduced in size.

We suggest that group cognition lowered the demands for neural architechtures required to support some aspects of individual intelligence and decision making (Bailey and Geary, 2009). This effect may have become even more pronounced with the advent of writing ca. 5000 years ago (Schmandt-Besserat, 2010), which falls within the estimated 95% CI for the pronounced reduction in Holocene human brain size (**Figure 1**). During human history, social groups became larger, social interactions more frequent, social networks more complex, and tracking relationships more demanding (Bailey and Geary, 2009; Foley and Gamble, 2009). A rise in sociocultural complexity was not due to particular individuals becoming more intelligent and culturally skilled, but because of the emergence of collective intelligence resulting from a growing population of interconnected humans and interacting human groups. As group size increases, interactions with a dynamic and exceedingly complex social landscapes result in increased demands on the brain (Bickart et al., 2011; Kanai et al., 2012). However, because of the metabolic demands of the brain (Pontzer et al., 2016), there may be limits to feedback loops between social network size and brain structure. If group decision-making generated adaptive group responses exceeding the cognitive accuracy and speed of individual decisions and had a fitness consequence, then human brain size may have decreased as a consequence of metabolic cost savings.

Population size expanded dramatically with the advent of agriculture, beginning ∼10 kyr and grew exponentially from an estimated five million to over 100 million by 3000 years ago (Goldewijk et al., 2011). This increase in population coincided with deterioration in individual health (Armelagos et al., 1991; Milner, 2019) and increases in infection rate (Eshed et al., 2010), pathogenic load (Page et al., 2016), and virulence (Menneret et al., 2010). It remains possible, then, that the high energetic cost of a heightened immune response (Wells and Stock, 2020), might have been a factor in Holocene brain reduction. In fact, Crabtree (2013a; 2013b) proposed this immunity-for-intelligence trade-off in his controversial "Idiocracy Hypothesis," though this idea has been criticized on the basis of flawed assumptions (Kalinka et al., 2013; Mitchell, 2013).

Gowdy and Krall (2013) draw parallels between the ultrasocial human superorganism, complete with division of labor and "economic organization around surplus" that arose in the Holocene and the sociobiology of agricultural eusocial insects, including some ants and termites. Brain size reduction occurred in traditional hunter-gatherer human populations (Wiercinski, 1979) that never adopted sedentary agricultural practices, but have complex social networks (Apicella et al., 2012). Foster and Ratnieks (2005) suggest that the presence of post-reproductive female helpers (grandmothers) universally present in human societies is sufficient to characterize humans as a "new eusocial vertebrate," offering additional support for the value of broad comparisons across unrelated taxa.

CONCLUSION

We suggest that patterns of human brain evolution were influenced by collective intelligence, a convergent characteristic of diverse group-living animals (Surowiecki, 2004; Sumpter, 2006; Woolley et al., 2010; Morand-Ferron and Quinn, 2011; Reid et al., 2015; Biro et al., 2016; Bates and Gupta, 2017; Sasaki and Biro, 2017). The precise role of societal information flow, distribution, and transfer as emergent group properties that may affect brain evolution and neural functioning is not well understood (e.g., Weaverdyck and Parkinson, 2018). Large brains may not be required to generate complex behavior, and brain mosaicism and circuitry—rather than overall size—may be important (Healy and Rowe, 2007, 2013; Chittka and Niven, 2009; Avarguès-Weber et al., 2018; Logan et al., 2018; Godfrey and Gronenberg, 2019). Computational models (e.g., Feinerman and Traniello, 2016; Reséndiz-Benhumea et al., 2021) and patterns in some ant clades (Muscedere and Traniello, 2012; Riveros et al., 2012; Muratore et al., 2021) suggest that group-level cognition may select for reduced brain size and/or adaptive brain size variation. Moreover, complex systems theory predicts that greater social complexity derives from individual simplicity (Delgado and Solé, 1997), although the neurobiological and behavioral meaning of "simplicity" is unclear. Complexity in eusocial insect colony organization may involve selection for either smaller, neurally differentiated worker brains (Lihoreau et al., 2012; Riveros et al., 2012; O'Donnell et al., 2015; Feinerman and Traniello, 2016) or larger brains (Wehner et al., 2007) able to metabolically offset increased production and operation costs (Kamhi et al., 2016). It seems unlikely that a "theory of theories" will appear to universally and meaningfully explain the multiple roles sociality can play across taxa as evolutionarily as divergent as humans and ants. We advocate for an ecumenical and open-minded approach that integrates theories of social, sociocultural, ecological, and mosaic and metabolic brain evolution to create an awareness of the breadth of the natural landscape of possibilities that can encompass both brain size increase and reduction. Human/ant comparisons have heuristic value and can offer a conceptual compass to guide future research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JD and JT conceived the study and wrote the manuscript. JD accumulated the hominin brain dataset. LF ran the change-point analysis and produced **Figure 1**. JD and JT edited the manuscript, with contributions from AC and LF. All authors edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.742639/full#supplementary-material

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Olfactory System Morphology Suggests Colony Size Drives Trait Evolution in Odorous Ants (Formicidae: Dolichoderinae)

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In social insects colony fitness is determined in part by individual worker phenotypes. Across ant species, colony size varies greatly and is thought to affect worker trait variation in both proximate and ultimate ways. Little is known about the relationship between colony size and worker trait evolution, but hypotheses addressing the role of social structure in brain evolution suggest workers of small-colony species may have larger brains or larger brain regions necessary for complex behaviors. In previous work on odorous ants (Formicidae: Dolichoderinae) we found no correlation between colony size and these brain properties, but found that relative antennal lobe size scaled negatively with colony size. Therefore, we now test whether sensory systems scale with colony size, with particular attention to olfactory components thought to be involved in nestmate recognition. Across three species of odorous ants, *Forelius mccooki*, *Dorymyrmex insanus*, and *D. bicolor*, which overlap in habitat and foraging ecology but vary in colony size, we compare olfactory sensory structures, comparing those thought to be involved in nestmate recognition. We use the visual system, a sensory modality not as important in social communication in ants, as a control comparison. We find that body size scaling largely explains differences in eye size, antennal length, antennal sensilla density, and total number of olfactory glomeruli across these species. However, *sensilla basiconica* and olfactory glomeruli in the T6 cluster of the antennal lobe, structures known to be involved in nestmate recognition, do not follow body size scaling observed for other structures. Instead, we find evidence from the closely related *Dorymyrmex* species that the larger colony species, *D. bicolor*, invests more in structures implicated in nestmate recognition. To test for functional consequences, we compare nestmate and non-nestmate interactions between these two species and find *D. bicolor* pairs of either type engage in more interactions than *D. insaus* pairs. Thus, we do not find evidence supporting a universal pattern of sensory system scaling associated with changes in colony size, but hypothesize that observed differences in the olfactory components in two closely related *Dorymyrmex* species are evidence of a link between colony size and sensory trait evolution.

Keywords: antennal sensilla, ommatidia, antennal lobe glomeruli, social interactions, pheromone GC-MS

INTRODUCTION

Superorganisms represent an increase in biological complexity from solitary organisms, making them a common focus of complexity studies (Cole, 1985; Bonner, 1993; Szathmáry and Maynard Smith, 1995; Bourke, 1999; Anderson and McShea, 2001; Jeanson et al., 2012; Kennedy et al., 2017). In social insects the colony is the reproductive unit of the superorganism and complexity may scale with colony size in a manner similar to complexity scaling with body size or group size across other taxa (Bonner, 1993). Indeed, across ant species, reproductive dimorphism (Bourke, 1999), worker polymorphism (Bonner, 1993; Murakami et al., 2000), and division of labor (Jeanson et al., 2007; Ferguson-Gow et al., 2014) are correlated with colony size. Importantly, components of increased complexity, e.g., morphological (Tschinkel, 1988; Thomas and Elgar, 2003) and behavioral (Jeanson et al., 2007; Ferguson-Gow et al., 2014) differentiation in a colony, sometimes arise only after colonies reach a certain size (Dornhaus et al., 2011).

Studies addressing the role of social structure in nervous system trait evolution often propose that social complexity, generally measured by colony size, will be negatively correlated with individual worker behavioral complexity (Anderson and McShea, 2001; Gronenberg and Riveros, 2009; O'Donnell et al., 2015) and hypothesize that relative brain investment, particularly in brain regions associated with more complex behaviors such as multi-modal learning and memory, will decrease with increasing colony size (Riveros et al., 2012; O'Donnell et al., 2015; Kamhi et al., 2016). However, individual workers of social species often show behavioral and cognitive skills comparable to solitary relatives (Gruter et al., 2011; Pasquier and Grüter, 2016; Hollis et al., 2017; Yilmaz et al., 2017), and comparisons seeking to link colony size with changes in brain structure may be complicated by confounding variables such as habitat differences or phylogenetic distance (Kamhi et al., 2016; Godfrey and Gronenberg, 2019b). Furthermore, complex collective behaviors may emerge from expanded communication systems or require relatively small changes in neural circuitry (Lihoreau et al., 2012; Bouchebti and Arganda, 2020) without changes to individual behavioral complexity (Jeanson et al., 2012; Feinerman and Korman, 2017). Thus, our understanding of the role of system complexity in individual-level trait evolution will be aided by comparisons of individuals from closely related species that vary in mature colony size but overlap in other drivers of trait evolution such as habitat and foraging ecology (Godfrey and Gronenberg, 2019a).

The evolution of larger social groups is hypothesized to act as a unique driver of trait evolution, particularly in traits related to intraspecific communication and recognition of group members (Stuart, 1991; Bourke, 1999; Freeberg et al., 2012). In solitary organisms, intraspecific communication and kin recognition are important to coordinating reproductive and familial relationships; in social organisms these same systems may be expanded or modified for interactions among individuals of varied relatedness. Since the evolution of sociality involves an expansion of the type and number of relationships among conspecifics, it represents an increase in biological complexity from solitary life histories (McShea, 1996). Similarly, the number and type of interactions may also scale with group size across social species and have consequences for individual traits (Anderson and McShea, 2001), particularly those related to intraspecific recognition and communication (Dunbar, 1992).

Superorganisms are colonies, not societies, with important differences in intragroup recognition and communication. Rather than individualized recognition of group members (Wells et al., 2003), superorganismal species are thought to use a general recognition system that allows them to assess whether an individual is a nestmate or non-nestmate (Breed, 2014; Esponda and Gordon, 2015). Intraspecific communication in Hymenoptera is largely chemosensory and mechanosensory in nature (Hölldobler, 1999), with the origins of chemical signaling in social insects originating from those used in defense (Mitra, 2013), fertility (Van Oystaeyen et al., 2014; Oi et al., 2015) and kin recognition (Lihoreau et al., 2007) by solitary species. Chemosensory information is processed primarily in the antennal lobe, and the diversification of signaling in Hymenoptera is mirrored by a notable expansion in olfactory receptor genes (Zhou et al., 2015), and increased complexity of olfactory system morphology (Dacks and Nighorn, 2011; Rössler and Zube, 2011).

In ants, social signal reception occurs primarily through sensilla on the antennae and maxillary palps. The *sensilla basiconica* have been identified as important in nestmate recognition in ants (Ozaki et al., 2005; Nishikawa et al., 2012; Sharma et al., 2015). These stout, pegged sensilla are set in a small indentation in the cuticle (Hashimoto, 1990; Renthal et al., 2003) and, while originally described as having a single apical pore (Hashimoto, 1990), but are now known to be multiporous (Sharma et al., 2015). Most other types of sensilla house a small number of sensory neurons, but each *s. basiconicum* can be innervated by more than 100 sensory neurons (Nakanishi et al., 2009). Olfactory sensory neurons (OSNs) expressing a particular odorant receptor complex converge on the same glomerulus (synaptic cluster) in the antennal lobe such that the number of glomeruli is often a good estimate of odorant receptor (OR) genes (Hansson and Stensmyr, 2011; Haverkamp et al., 2018; but see Younger et al., 2020). In ants, OSNs from the *s. basiconica* form glomeruli in a cluster called T6, suggesting this region plays a role in nestmate recognition (Ozaki et al., 2005; Nakanishi et al., 2009; D'Ettorre et al., 2017) and may be subject to selection on social communication. On the other hand, while some ants have elaborate visual systems used in navigation, foraging, and learning and memory (Jaffé et al., 1990; Narendra et al., 2011; Yilmaz et al., 2017, 2019; Fernandes et al., 2018; Wehner, 2020), and may rely on multiple modalities for nestmate recognition (Bos et al., 2010), there are no documented examples of visually based nestmate recognition systems in ants (Hölldobler, 1999).

With approximately 900 described species, Dolichoderinae is one of the four largest, species-rich subfamilies of ants (Ward et al., 2010). They are commonly referred to as odorous ants, a moniker referencing the volatile compounds reminiscent of fermented cheese or rotting fruit (Penick and Smith, 2015) emitted from their pygydial (anal) gland (Wheeler et al., 1975). However, the species diversity of dolichoderine ants is not reflected in relative research interest in dolichoderine ant biology, where the majority of studies focus on the invasive pest Argentine ant (*Linepithema humile*). In the experiments described here, we ask if differences in sensory systems across workers of three species of odorous ants in the tribe Leptomyrmecini—*Dorymyrmex bicolor*, *D. insanus*, and *Forelius mccooki* (Formicidae, Dolichoderinae; **Figures 1**, **2A,B**)—are explained by body size scaling and if not, whether variation in colony size better explains differences. Importantly, these species overlap in habitat and foraging ecology, even competing with each other for resources (Bestelmeyer, 2005), commonalities leveraged here to control for differences in sensory ecology driven by these variables. We predict that variation in visual sensory systems, presumably marginally related or unrelated to social communication in these species, will be explained largely by body size. In contrast, given that the olfactory system supports social communication in ants, its evolution should be influenced by or influence colony size evolution. We therefore expect differences in the olfactory system, particularly in structures related to nestmate recognition, to correlate better with colony size than body size.

MATERIALS AND METHODS

Species Identification and Collection

Forelius and *Dorymyrmex,* two sister dolichoderine ant genera known for their xerophilic and thermophilic habits, also have in common a tendency toward remarkable intraspecific variation. There are currently three species of *Forelius* in the United States, *F. damiani* (Guerrero and Fernández, 2008) (known in the United States only from southern Texas), *F. pruinosus* (Roger, 1863) and *F. mccooki* (McCook, 1880). The last two species vary significantly in color and hardly at all in morphology; few features can reliably separate them. The primary distinction is one of standing pilosity: *F. mccooki* has erect setae on the scapes, posterior margin of the head, and external face of the tibiae, while *F. pruinosus* has few to none of these standing hairs—but even this character has occasional intermediates (Ward, 2005). The small yellow *Forelius* abundant in Tucson, Arizona that were collected for our study have numerous standing setae on the aforementioned structures, so we identify these samples as *F. mccooki.* Some researchers have found preliminary evidence of two *Forelius* clades throughout the western United States, but also found conflicting results that suggest *F. mccooki, F. pruinosus,* and an undescribed orange *Forelius* sp. are tangled together in a single clade (Cover, pers. comm., 2021). We concur with Ward (2005) and Fisher and Cover (2007) that North American *Forelius* taxonomy needs further study.

The taxonomy of North American *Dorymyrmex* is notoriously complicated (Creighton, 1950; Snelling, 1995; Deyrup, 2017). An ongoing phylogenetic analysis and taxonomic revision has confirmed that species delimitation, particularly based on the worker caste, is extremely difficult in this group (Oberski, in press) and there are a number of species in western North America that have yet to be described (Fisher and Cover, 2007). Three *Dorymyrmex* species undoubtedly found in Arizona are *D. bicolor* (Wheeler, 1906), *D. insanus* (Buckley, 1866), and *D. wheeleri* (Kusnezov, 1952). *D. wheeleri* is a small, rather distinctive *Dorymyrmex* species, and although its type locality is Tucson, Arizona—the source of our colonies for this study—no confirmed collections of the species exist beyond the type series. Both *D. insanus* (type locality Howard Co., Texas) and *D. bicolor* (Maricopa Co., Arizona) are conspicuously present across the south-central and southwestern United States and northern Mexico. However, there are other species known from neighboring states whose range may very well extend into Arizona: *D. flavus* (McCook, 1880) and *D. smithi* (Cole, 1936) have been recorded from New Mexico; *D. paiute* (Snelling, 1995), southern Utah; and *D. lipan* (Snelling, 1995), west Texas (Snelling, 1995; Mackay and Mackay, 2002). To confirm our hypothesis that the dark and bicolored *Dorymyrmex* samples we collected are *D. insanus* and *D. bicolor,* we first checked Snelling's (1995) literature review and key to United States *Dorymyrmex* based on workers, which is partially recounted below. Morphometrics include head length (HL), head width (HW), cephalic index (ratio of HW/HL) (CI), eye length (EL), and interocular distance (IOD).

> Snelling (1995): Head relatively narrow, CI usually less than 88, rarely up to 90. Vertex of head straight or slightly convex. Eye relatively large, IOD usually less than 1.5 × EL. Propodeal tubercle relatively prominent. Pronotum usually with discal seta pair. Color light to dark brownish, head and gaster commonly darker than mesosoma. (Kansas to central Texas, west to southern California)
>
> = *insanus* (Buckley, 1866)
>
> Snelling (1995): Head relatively broad, CI over 90. Vertex of head usually distinctly concave in frontal view, rarely straight. Eye relatively small, IOD at least 1.75 × EL. Head and mesosoma red. (Western Texas to southern Nevada and California)
>
> = *bicolor* (Wheeler, 1906)

Our Tucson collections of *Dorymyrmex* each conform to one of these two descriptions, with the exception of the cephalic index measurements of *D. insanus*—even those of the neoparatype series, which contradict Snelling's key. Our *D. bicolor* and *D. insanus* can also be differentiated by worker body size; *D. bicolor* individuals are larger than *D. insanus* (HW 0.84–1.00 mm vs. 0.73–0.81 mm; **Supplementary Figure 1**), although this trend may weaken with larger sample sizes. Among these populations, species-level mean head width corresponds with body size measured by mass (Godfrey and Gronenberg, 2019b); thus, head width is used as a proxy for body size throughout this study, although it may not hold true in other locations or with different species of *Dorymyrmex.*

Across *D. bicolor* colonies, consistent differences appear to create two morphotypes (**Supplementary Figure 2**). Some measurements distinguish these *D. bicolor* morphotypes quite well, such as the ratio of eye size to head width (**Supplementary Figure 1B**) and body size (DPL, DF)

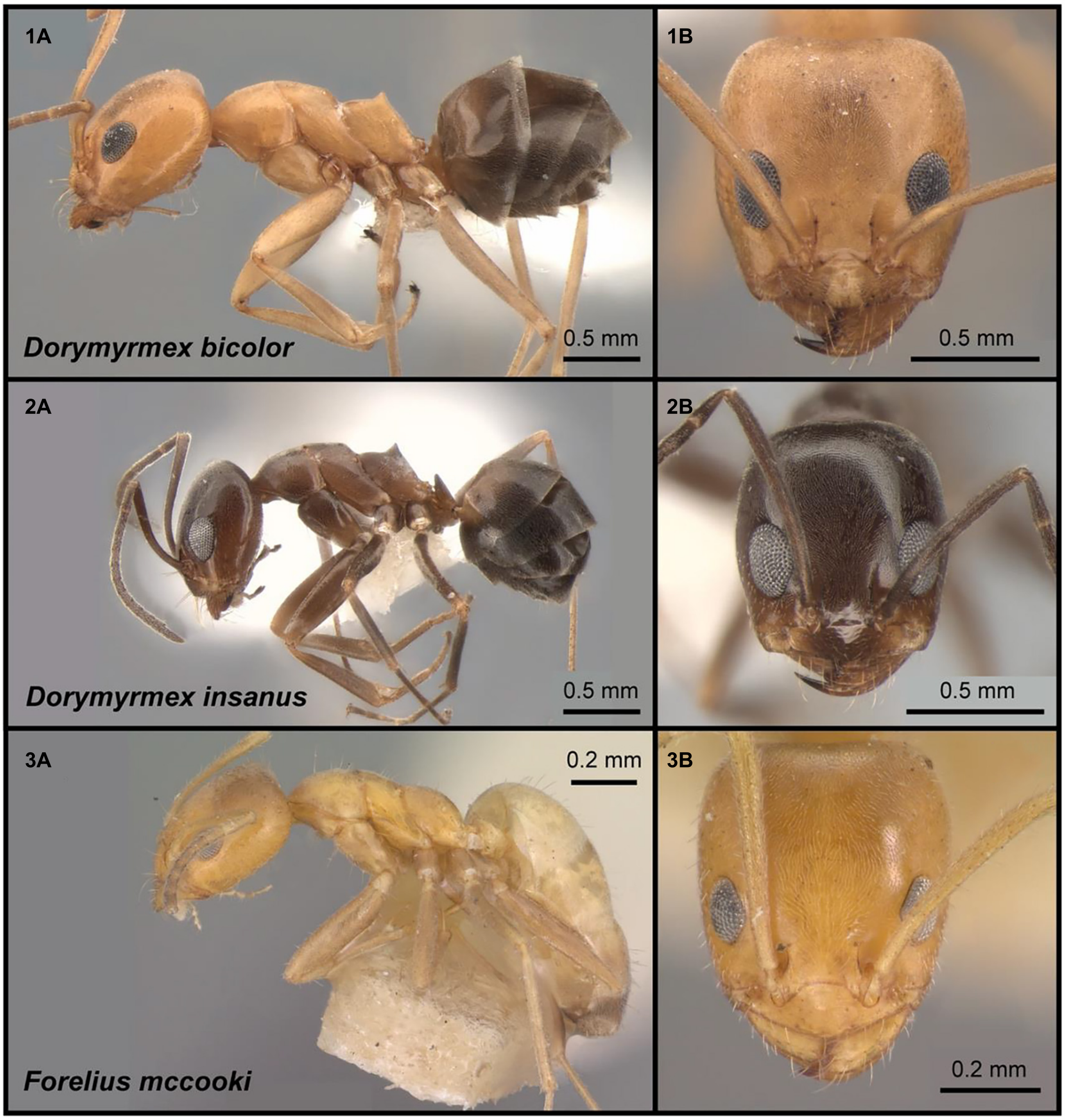

FIGURE 1 | Three sympatric species of dolichoderine ants at the focus of the present work. **(1A,B)** *Dorymyrmex bicolor*, specimen CASENT0841125. **(2A,B)** *Dorymyrmex insanus*, specimen CASENT0841126. **(3A,B)** *Forelius mccooki*, specimen CASENT0102754. Complete specimen data and images are hosted on AntWeb (www.antweb.org); photographers Jill Oberski (1,2), Jen Fogarty (3).

(**Supplementary Figures 2D,E**), while others show all *D. bicolor* samples as a single undifferentiated cluster, such as cephalic index (**Supplementary Figure 2F**) or scape length (**Supplementary Figure 2E**). Variation in allometry and color seem consistent at the colony level, and thus may indicate simple variation at the level of the reproductive unit or perhaps differences in colony age or some other unidentified factor. Ultimately, for the purposes of the present work, we treat our *D. bicolor* samples as a single species.

To ease future taxonomic efforts, we have also selected a representative voucher specimen for each species and morphotype. These specimens have been deposited at the University of California Davis insect collection (UCDC) with the following unique identifiers: *D. bicolor* morphotype 1, CASENT0841125; *D. bicolor* morphotype 2, CASENT0841124; *D. insanus,* CASENT0841126; *F. mccooki,* CASENT0841127.

Colony size rankings are based on previously reported measurements of workers outside the nest during peak foraging times (Godfrey and Gronenberg, 2019b). Based on these observations *D. insanus* was considered to have small colonies ($\bar{x}$ = 2, s = 1.5 workers outside nest); *D. bicolor,* intermediate sized colonies ($\bar{x}$ = 14, s = 7.3); and *F. mccooki,* large colonies ($\bar{x}$ = 46, s = 14; Godfrey and Gronenberg, 2019b). For the current study, individual workers observed foraging at regularly monitored colonies in Tucson, Arizona and adjacent municipalities (**Supplementary Table 1**) were collected live into

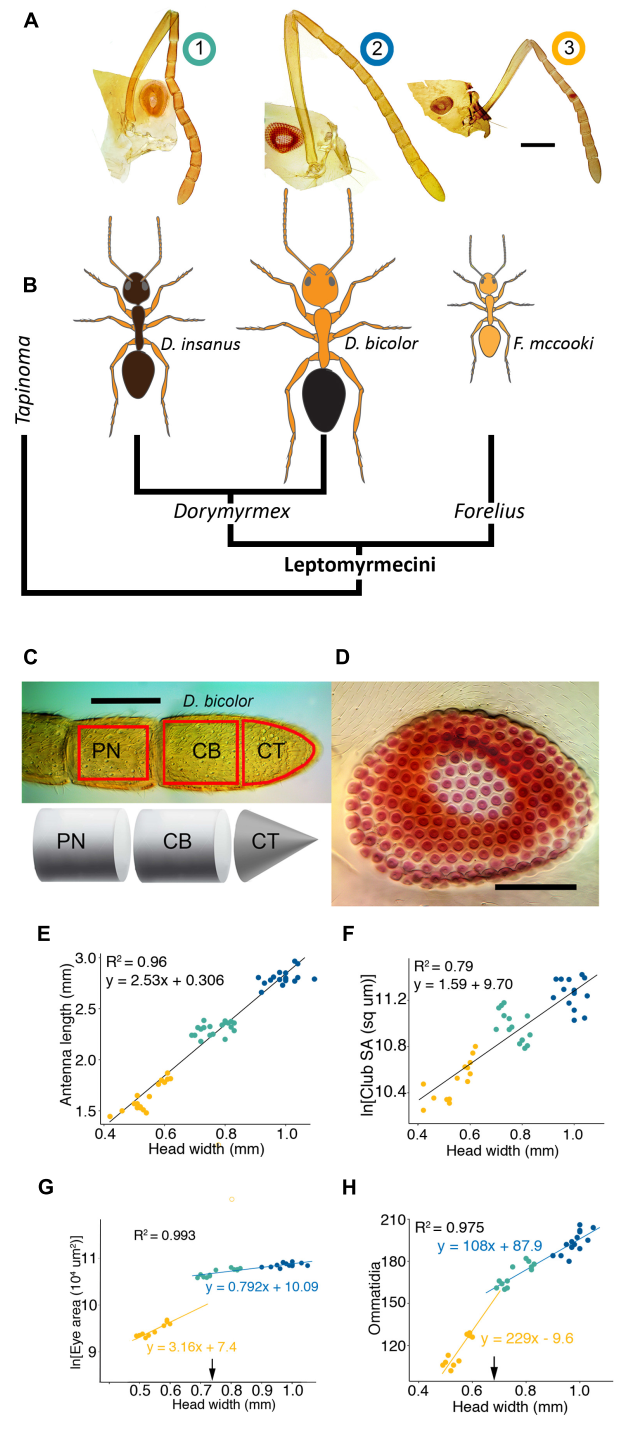

FIGURE 2 | External sensory structures scale with body size. **(A)** Microphotographs of parts of the head capsule including the eye and antenna of the three focal species arranged by colony size indicated by number rankings above **(B)**. **(B)** Phylogenetic relationships among dolichoderine ants included in this study. **(C)** Sampling area for the three antennal regions (top; red boxes) and diagram of measurements taken for surface area (bottom); penultimate segment = PN; club base = CB; club tip = CT. **(D)** Photomicrograph of the eye of *D. bicolor*; to show ommatidia; eye surface area measured as footprint of eye on head capsule.

(Continued)

FIGURE 2 | (Continued)
Scale bars in **(A,C,D)** = 100 μm. Differences in antennal length **(E)** and antennal surface area **(F)** are explained largely by differences in body size. Eye area **(G)** and Ommatidia numbers **(H)** scale with body size, but significant differences exist between *Forelius* and the *Dorymyrmex* species and scaling is best described using piecewise regression (yellow and blue lines, respectively) using head width of 0.69 mm as a breakage point (black arrow). For **(G,H)** R-squared from piecewise linear regression indicated in black. Slopes for piecewise regression indicated in colors that correspond to lines above and below breakage point. Color code for **(B,E–H)**, this figure and **Figures 3**, **4**: *F. mccooki* yellow; *D. insanus* cyan; *D. bicolor* blue.

Falcon 50 mL conical centrifuge tubes (Corning, #352070) using an aspirator and transported back to the lab for experiments.

External Sensory Morphology

To count sensilla and ommatidia, head capsules were cleared in 30% hydrogen peroxide for 1–3 days, rinsed, incubated in 80% glycerol, mounted on a slide with a polyvinyl alcohol mounting medium, Mowiol® 4–88 (Sigma-Aldrich), and covered with a #1.5 coverslip. Antennae and eyes were imaged in brightfield with a SpotFlex camera (FX1500WS, Diagnostic Instruments, Inc., Sterling Heights MI, United States) mounted on a Zeiss Axioplan microscope. Entire antennae were imaged using a 2.5× objective, eyes using a 20× or 40× objective, and antennae segments using a 63× objective (**Figure 2A**). Images were captured with SPOT Basic image software (Diagnostic Instruments, Inc., Sterling Heights MI, United States). Antenna length, eye size, ommatidia number and sensilla density were measured from stacks of images in Fiji (Schindelin et al., 2012). All measurements were averaged over the two sides to produce individual-level measurements for each sensory structure for statistical analysis. Some head capsules were particularly fragile following clearing and, in these cases, only one antenna or eye was available for analysis.

The surface area (SA) of antennae segments was approximated from measurements of segment diameter and height (**Figure 2C**). The penultimate segment was approximated as a cylindrical tube with $SA_{pen} = 2\pi rh$. Surface area of the apical club segment (SA_{club}) was approximated as a cylindrical tube for the proximal two thirds of its height and the lateral surface of a cone for the distal most third such that

$$SA_{\mathrm{club}} = (\pi r_1 + \pi r_2)\left(\sqrt{(r_1 + r_2)^2 + \left(\frac{2}{3}h\right)^2}\right) + \pi r_2\sqrt{\left(r_2^2 + (\frac{1}{3}h)^2\right)}$$

(**Figure 2C**). Eye area was measured as the traced boundary of the eye on the cuticle (**Figure 2D**). Ommatidia were counted using the Cell Counter plugin in Fiji (De Vos, 2001).

To quantify sensilla density, three polygons were drawn along the antenna, one on the penultimate segment, one on each of the base and tip of the club (**Figure 2C**). Depending on segment size and shape, polygons sampled from 20 to 30% of the approximate total surface area of the segment (**Supplementary Figure 4A**). All sensilla with their base inside the sampling polygon were counted. Sensilla density was averaged over segment and antennae. *Sensilla* *basiconica* (*s. basiconica*) were identified as stout, pegged sensilla with a blunt, terminal end (Nakakuki, 1986; Renthal et al., 2003; Ramirez-Esquivel et al., 2014) which protrude from the antenna surface at a more obtuse angle than other sensilla (indicated by red arrows in **Figure 3A**) and are easily distinguished using brightfield microscopy (Kelber et al., 2010).

Antennal Lobe Morphology

To quantify differences in the number of olfactory glomeruli, brains were labeled with an α-synapsin antibody to visualize glomeruli. Brains were dissected in phosphate-buffered saline (PBS) and microwave-fixed in 4% paraformaldehyde in PBS (low power at 18°C under vacuum for two cycles of 2 min), then left in fixative for 12 h at room temperature. Following blocking with 2% normal goat serum (Thermo Fisher Scientific # 31872), brains were permeabilized with 1% Triton X-100 in PBS (Electron Microscopy Supply, Fort Washington, PA; PBS-TX), rinsed with 0.1% PBS-TX, and incubated on a shaker at 25°C for two nights in primary antibody (1:500 in 2% goat serum in 0.2% PBS-TX). Monoclonal Drosophila synapsin I antibody (SYNORF1, AB_2315426; Developmental Studies Hybridoma Bank #3C11) was used as the primary antibody to label synapsin. Subsequently, brains were washed in 0.1% PBS-TX and incubated overnight at room temperature in Alexa Fluor 568 (AB_2534072, Thermo Fisher Scientific #A-11004) goat anti-mouse secondary antibody (1:100 in PBS) on a shaker. After secondary incubation, brains were washed in 0.1% PBS-TX and rinsed with distilled water before being dehydrated in increasing concentrations of ethanol in distilled water (10 min each in 50, 70, 80, 95, 100, 100%) and mounted in custom-made aluminum well slides with #1.5 coverslips. Brains were cleared by incrementally removing ethanol and replacing it with methyl salicylate. Brains were imaged on an inverted Zeiss 880 Laser Scanning Confocal Microscope using a plan-Apochromat 20× 0.8 aperture objective and optically sectioned in the horizontal plane at 1-micron intervals. Section thickness was corrected by a factor of 1.64 (adjusted section thickness = 1.64 microns) to account for the refractive index mismatch between air and methyl salicylate (Bucher et al., 2000).

To visualize olfactory sensory neuron (OSN) tracts and glomerular clusters in the antennal lobe, mass fills of OSNs were performed. For these experiments workers were anesthetized on ice and the club or last three segments of their antennae were removed with surgical scissors. A small crystal of Dextran, Texas Red, 3,000 MW was dissolved in physiological saline (130 mM NaCl/5 mM KCl/4 mM $MgCl_2$/5 mM $CaCl_2$/15 mM Hepes/25 mM glucose/160 mM sucrose, pH 7.2; Groh et al., 2004), allowed to dry until sticky, and placed on the excised tip. Ants were allowed to recover in humidified chambers with sucrose (30% w/w) for 2–4 days until anesthetized and euthanized. Brains were fixed in 2% glutaraldehyde, 2% paraformaldehyde for 24 h at room temperature, then rinsed, dehydrated, mounted, and imaged as described for whole mount synapsin labeling.

Whole-brain images were manually segmented using the TrakEM2 software package in Fiji (Cardona et al., 2012). Volumes of histologically recognizable subunits, the glomeruli, were traced in one or both hemispheres in 4–6 individuals of each species. Because basiconic sensilla innervate the T6 cluster of glomeruli (Kelber et al., 2010; Nakanishi et al., 2010), glomeruli of this cluster were identified from segmented images following tracing of all glomeruli in order to look for differences that

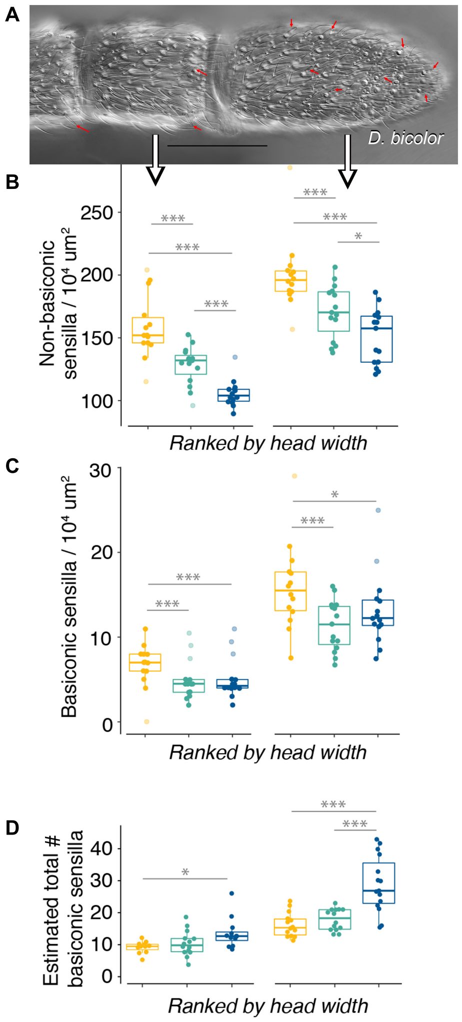

FIGURE 3 | Basiconic sensilla density specifically does not scale with body size. **(A)** Microphotograph (63× oil immersion lens) of distal antennal segments [penultimate (left) and club (right)] of *Dorymyrmex bicolor*; red arrows point to basiconic sensilla; scale bar = 100 μm; large arrows point at the respective data sets in **(B,C)**. **(B)** Non-basiconic sensilla density decreases with increasing body size on both the penultimate and club segments. **(C)** Basiconic sensilla density does not decrease with increasing body size, specifically in the comparison of *Dorymyrmex* species. **(B,C)** Show sensilla density for the penultimate (left) and club (right) antennal segments; outliers excluded from analysis shown with decreased saturation. **(D)** Estimated total number of basiconic sensilla when mean basiconic sensilla density is multiplied by mean surface area for each segment (penultimate, left; club, right) for individual ants; data shown does not include outliers. Statistical comparisons of sensilla density across species made using a generalized linear model with a quasi-Poisson distribution followed by ANOVA and *post hoc* comparisons using Tukey's Honestly Significant Difference. $^{*}p < 0.05$; $^{***}p < 0.001$. Color code for **(B–D)**: *F. mccooki* yellow; *D. insanus* cyan; *D. bicolor* blue.

correspond with differences in basiconic sensilla across taxa. The T6 cluster was identified based on location description and images from the myrmicine ant, *Atta vollenweideri* (Kelber et al., 2010) and the formicine ant, *Camponotus japonicus* (Nakanishi et al., 2010; Nishikawa et al., 2012). Brains were not always imaged in the same plane and, while it is possible to count all glomeruli from any plane, it was difficult to distinguish T6 glomeruli in images taken outside the dorsoventral (neural axis) plane. Therefore, to ensure T6 glomeruli could be appropriately quantified, four antennal lobes from each of the focal species for this analysis, *D. bicolor*, *D. insanus*, and *F. mccooki*, were chosen based on image quality and orientation.

Potential Pheromone Compounds

Because *D. bicolor* showed significantly greater estimated total *s. basiconica* (**Figure 3D**) than the small-colony relative, *D. insanus*, and because differences in glomeruli number between these species could be explained largely by those in the T6 cluster (**Figures 4A,B**), we asked if the number of compounds we could detect through gas chromatography/mass spectroscopy (GC-MS) from *D. bicolor* was greater than from *D. insanus*, with a particular interest in putative cuticular hydrocarbons that are in part detected by *s. basiconica*. Eight ants from a single colony of each species were used in the analysis. The gaster contents and bodies were analyzed separately with the expectation that cuticular hydrocarbons would show up in both samples, whereas many of those produced for alarm and recruitment would be unique to the metasoma ("gaster"). Ants were anesthetized on ice and the gaster excised from the head and remaining thorax segments. Compounds were eluted in the following manner: the head and thorax from the 8 ants were placed in one 2 ml glass vial and soaked with 500 μL dichloromethane (DCM; check supplier) for 15 min, and gasters were placed in a different 2 ml vial with 500 μL DCM and crushed using a tissue grinder. A control vial of 500 μL DCM was prepared at the same time. These procedures were carried out in a lab hood and all tools, vials, and lids were rinsed three times in DCM prior to use. Chemical analysis of *Dorymyrmex* compounds was carried out at the Analytical & Biological Mass Spectrometry facility at the University of Arizona using a Shimadzu SHRXI GC column. The eluent was introduced into a Shimadzu QP2010S mass spectrometer and ionized by electron impact. Identification of peaks was accomplished by comparison to the National Institute of Standards and Technology (NIST) mass spectral library (Shen et al., 2016). Existing reports of gland contents in the literature were consulted to determine potential gland sources of compounds identified from *D. bicolor* and *D. insanus*. For detailed methods, see **Supplementary Material**.

Behavior

To test whether the difference in total *s. basiconica* between *D. insanus* and *D. bicolor* is correlated with behavior, we recorded and scored interactions between pairs of nestmates and non-nestmates for each species. Ants were collected from nest entrances between 08:00 and 10:00 and stored in a cool chamber to be transported to the lab. Once in the lab ants were stored in fluon-lined plastic boxes and given 30 min to acclimate before pairings occurred. Nestmate or non-nestmate pairs were placed in a 9 cm KIMAX® borosilicate glass petri dish on white lab paper and illuminated with a 75 W incandescent bulb surrounded by an aluminum diffuser and warmed to 30–32°C with a 1,500 W personal space heater (Holmes Products Corp., Milford, MA, United States) to mimic outdoor thermal conditions. Ants were filmed using a Sony HDR XR200 Handicam camera positioned on a tripod and focused down onto the petri dish. Ants were placed in the petri dish in succession and each was given 30 s to acclimate before either the addition of the second ant or the behaviors were coded. Ants were filmed for 3 min. Interactions were coded using the open-source event logging software, Behavioral Observation Research Interactive Software (**Figure 5A**, BORIS; Friard and Gamba, 2016). The following behaviors were coded: antennation (one or both ants tapping antennae on the other), grooming (one ant licking areas of the other ant's head or body; **Figure 5B**), trophallaxis, mandible flaring, biting (**Figure 5B**), retreating (when one ant moved quickly away from an interaction), chasing (when one ant moved quickly away from an interaction and the other followed), fighting (when ants were biting and wrestling occurred), and touch (fast or incidental contact that could not be coded as one of the other behaviors). Only behaviors that involved ants interacting were coded. Behaviors were scored as point events or state events, with state events including duration information. Behaviors scored as state events included antennation, grooming, and fighting. For the purpose of analysis, grooming and trophallaxis were considered affiliative behaviors, mandible flaring, biting, chasing, and fighting were considered aggressive behaviors, and touch and antennation were considered neutral behaviors.

Statistics

All statistics were conducted in R version 4.0.2 (R Core Development Team, 2020) using the R studio interface (R Studio Team, 2020) and the tidyverse (Wickham et al., 2019), lme4 (Bates et al., 2015), emmeans (Lenth, 2021), and ggpubr (Kassambara, 2020) packages. Hypothesis testing for scaling relationships in antennal length, club surface area, eye area, and ommatidia number were achieved with the construction of linear models (LMs) followed by ANOVA (**Supplementary Table 2**). Surface area measurements were natural log-transformed for these analyses. Tukey-adjusted least square means were used for pairwise comparisons. LMs that included head width alone were used to approximate slopes and intercepts for antennal length and club surface area (**Figures 2E,F**). Piecewise LMs with a break point at 0.69 mm were used to assess scaling in eye surface area and ommatidia number between *F. mccooki* and the *Dorymyrmex* species. Piecewise LMs estimate a single R-squared value and slopes for lines above and below the break point are presented. Differences in sensilla and ommatidia density across species were assessed using ANOVA on GLMs with quasi-Poisson likelihood. We tested whether sensilla density varied predictably with body size using the assumption that mean sensilla density should be significantly different in all pairwise comparisons of species in either an increasing or decreasing manner with body size. We detected significant

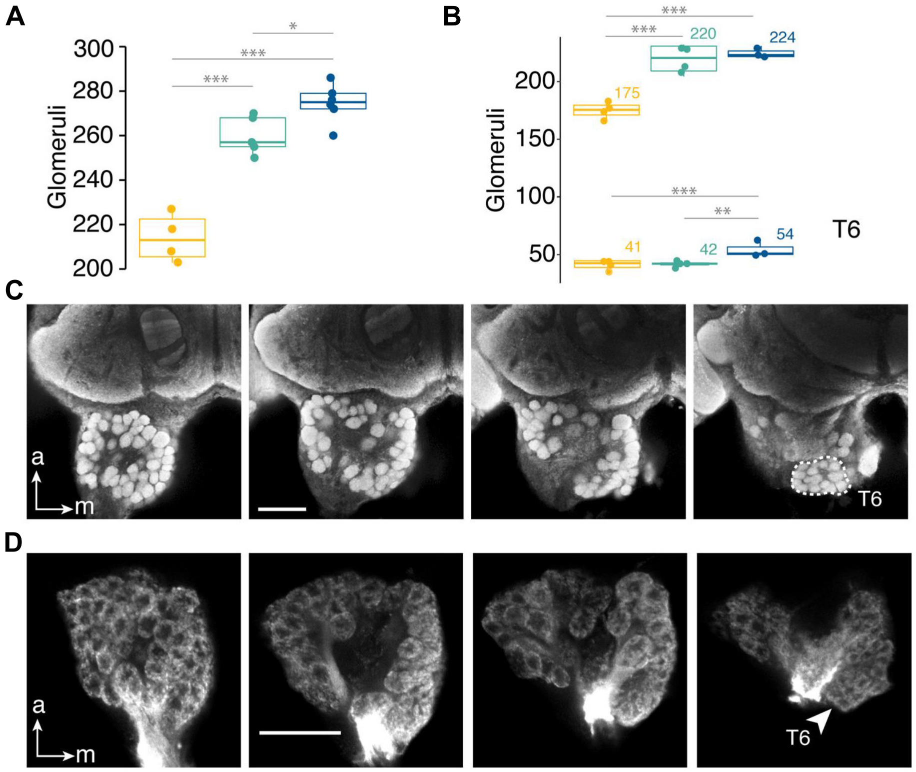

FIGURE 4 | Glomeruli in the T6 cluster account for differences in antennal lobe glomeruli between *Dorymyrmex* species. **(A)** Number of antennal lobe glomeruli. **(B)** glomeruli in the T6 cluster and other clusters in three species that vary in colony size. Number above boxes indicate mean glomeruli number for T6 cluster (bottom) and all other glomeruli (top). Species ranked in order of increasing mean head width along the x-axis. **(C)** Synapsin-labeled antennal lobe and **(D)** mass staining (dextran tracer) of antennal sensory neurons in the antennal lobe of *D. bicolor* with T6 cluster outlined **(C)** or indicated by arrowhead **(D)**, scale bars = 50 μm. Generalized linear model with gamma distribution and log link function used for statistical comparisons. Pairwise comparisons made using Tukey's honestly significant difference (HSD) test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

differences in sensilla density between segments ($\chi^2 = 124.885$, $df = 1$, $p < 0.001$), which is not part of our hypothesis structure and therefore segments were analyzed separately. We used GLMs with quasi-Poisson likelihood where head width was the predictor variable to determine the slope and fit of the relationship between body size and sensilla density across all species (**Supplementary Table 3**). Affiliative, aggressive, and neutral behavior events were compared using GLMs with Poisson likelihood followed by *post hoc* tests. Antennation durations were compared using the Mann-Whitney *U*-test across treatments within species.

RESULTS

Summary

Species rankings according to body size (**Figure 1**) do not correspond with species rankings according to colony size (**Figures 2A,B**, ranked smallest to largest colony size). This allowed us to assess whether sensory system scaling is explained by body size and whether patterns that are not associated with body size differences could be due to colony size differences. We found that gross morphological structures including antennal length (**Figure 2E**), surface area (**Figure 2F**), eye area (**Figure 2G**), ommatidita number (**Figure 2H**, **Supplementary Figure 3**), along with estimated total number of sensilla (**Supplementary Figure 4C**), and olfactory glomeruli (**Figure 4A**) scaled positively with body size. However, the total number of *s. basiconica* (**Figure 3D**) and glomeruli in the T6 cluster (**Figure 4B**), structures thought to play a role in social communication in ants, deviated from this pattern. While the density of total (**Supplementary Figure 4B**) and non-basiconic sensilla (**Figure 3B**) scaled negatively with body size, the density of *s. basiconica* did not and the large bodied, intermediate colony sized *D. bicolor* had *s. basiconica* density comparable to the medium body sized, small-colony *D. insanus* (**Figure 3C**). These sensilla innervate a cluster of the antennal lobe called T6 and coinciding with the *s. basiconica* findings, *D. bicolor* showed a greater number of T6 glomeruli than the other species (**Figure 4B**). In subsequent experiments to probe functional differences, we compared the closely related *Dorymyrmex* species and found no differences in the number of chemical compounds identified from bodies of workers (**Supplementary Figure 5** and **Supplementary Table 6**). However, we detected differences in the frequency and duration of social interactions, with *D. bicolor* individuals engaging

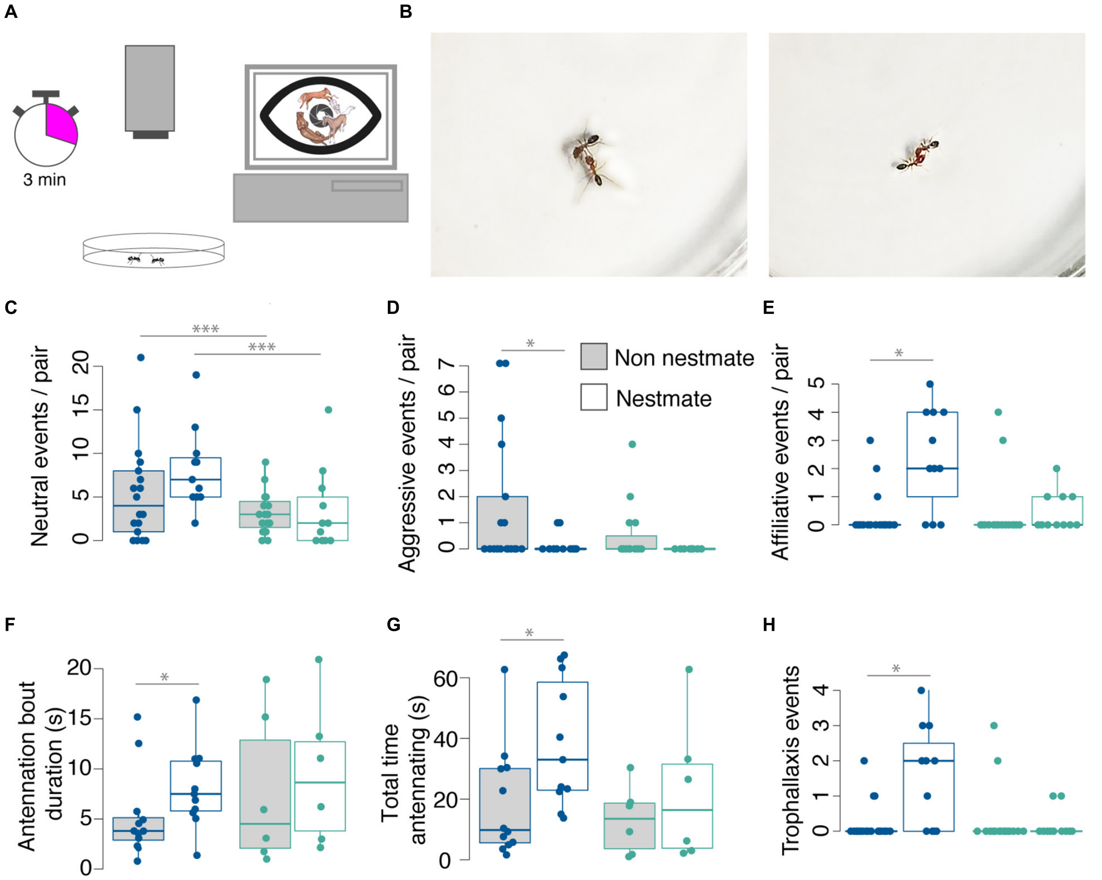

FIGURE 5 | *Dorymyrmex bicolor* engage in more social interactions than *D. insanus*. **(A)** Schematic of behavior method with pairs of ants filmed in a glass petri dish for 3 min and interactions coded in BORIS (Friard and Gamba, 2016). **(B)** Stills from recordings showing *D. bicolor* engaging in biting (left) and grooming (right) behaviors. In comparisons of nestmate interactions or non-nestmate interactions between species, *D. bicolor* interact more often than *D. insanus* **(C)**. In comparisons of nestmate and non-nestmate behavior within species, *D. bicolor* non-nestmates display significantly more aggressive interactions **(D)** and significantly fewer affiliative interactions **(E)** than nestmates, an effect not detected in *D. insanus*. *D. bicolor* nestmates participate in antennation for longer duration bouts **(F)** and a greater amount of total time than non-nestmates **(G)**; *D. insanus* nestmates and non-nestmates show comparable antennation bout duration and total time. **(H)** *Dorymyrmex insanus* display few trophallaxis events with no differences among nestmate or non-nestmate pairs, whereas *D. bicolor* nestmates engage in trophallaxis more frequently than non-nestmates. Statistical comparisons in **(A–E,H)** were made using a generalized linear model with a Poisson distribution followed by ANOVA and *post hoc* comparisons using Tukey's Honestly Significant Difference. Statistical comparisons in F and G were made using pairwise Mann-Whitney *U*-test between non-nestmate and nestmate pairs of a species. $*p < 0.05$; $***p < 0.001$; Color code for data point in **(C–H)**: *D. insanus* cyan; *D. bicolor* blue.

more in interactions with nestmates or non-nestmates than *D. insanus* (**Figure 5**).

External Sensory Morphology

Across species, antennal length and club surface area scaled positively with head width (HW; **Figures 2E,F**). However, in comparisons of club surface area, we detected a significantly greater club surface area in *D. bicolor* as compared with *D. insanus* when body size was taken into account (t-ratio = 2.511, $df = 38$, $p = 0.042$). Eye area and ommatidia number also scaled positively with head width (**Figures 2G,H**), but *F. mccooki* had significantly smaller eyes than would be predicted from the *Dorymyrmex* spp. regression line (**Figure 2G**) and steeper slope for ommatidia scaling with body size (**Figure 2H**), resulting in significantly greater ommatidia density in this species (**Supplementary Figure 3**).

Mounting antennae on glass slides provided little control over orientation; therefore, sensilla were sampled randomly from surfaces of antennae without a way of identifying whether the ventral or dorsal surface was sampled. Sensilla appeared to be somewhat stereotyped in their distribution in that similar patterns of sensilla at particular locations were recognizable across individuals. It is possible that density is not homogenous across the segment, particularly on the club, where the ventral surface is used frequently to probe objects in the environment. Even if sensilla distributions are non-homogenous, sampling was random and therefore sampling of two antennae seemed to provide a reasonable estimate of mean sensilla density, but variation in our estimates is somewhat large (**Figures 3B,C**), producing weak regression fits (**Supplementary Table 3**). *Forelius mccooki* samples were very fragile and often one of the two antennae was not of sufficient quality to count sensilla. Therefore, individual-level estimates from a single antenna were more common in this species and may explain greater variation in estimates for this species (**Figures 3B,C**; *F. mccooki*).

Non-basiconic sensilla density decreased with body size rankings on both the penultimate and club segments (**Figure 3B** and **Supplementary Table 3**). In all species, *s. basiconica* on the penultimate segment were located near the rostral edge (**Figure 3A**) and appeared to have a somewhat stereotyped pattern, occurring at regular intervals. During counting it appeared that density increased toward the tip of the club, with occurrences less apparently stereotyped than on the penultimate segment (**Figure 3A**). However, because the dorsoventral axis of the club could not be discerned from images, it is possible they are stereotyped along this axis. Density of *s. basiconica* did not scale as strongly with body size on either segment, as *F. mccooki* had densities greater than the two larger species, but *D. bicolor* had densities comparable to those of *D. insanus* (**Figure 3C**). In a generalized linear model using a pseudo-Poisson distribution, body size explained 49% of the variation in non-basiconic sensilla density on the club segment but only 12% of the variation in basiconic sensilla on this segment (**Supplementary Table 3**).

We hypothesized that differences in *s. basiconica* density may be due to changes in antennal surface area related to body size and, when surface area is taken into account, the total number of sensilla might still increase with body size across species. However, despite high *s. basiconica* density, *F. mccooki* had total sensilla numbers comparable to *D. insanus*, but fewer total sensilla than *D. bicolor* (**Figure 3D**) on both segments. In comparisons of *D. insanus* and *D. bicolor*, differences in total basiconic sensilla number were detected specifically on the club segment (**Figure 3D**), an effect likely driven by the combination of *D. bicolor* showing a larger than expected club surface area and comparable instead of lower *s. basiconica* density to *D. insanus*.

Antennal Lobe Anatomy

Somewhat unexpectedly, glomeruli number scaled positively with body size across species (c, t = 0.4.61, $df = 12$, $p < 0.001$; **Figure 4A**; *F. mccooki*, $\bar{x} = 214$, $SD = 10.7$; *D. insanus*, $\bar{x} = 260$, $SD = 8.6$; *D. bicolor*, $\bar{x} = 275$, $SD = 9.8$). Because estimated total *s. basiconica* number did not scale with body size, instead displaying a pattern suggesting either *D. insanus* had fewer *s. basiconica* than would be expected from its body size or that *D. bicolor* had many more, of particular interest was the difference in T6 glomeruli among species. The T6 glomerular cluster was identified in four antennal lobe samples from each of three species (**Figures 4C,D**), *F. mccooki*, *D. insanus*, and *D. bicolor*. Two of the *D. bicolor* antennal lobes used to identify T6 glomeruli came from the same individual and these values were averaged for statistical comparisons. *D. bicolor* had comparable mean numbers of non-T6 glomeruli to *D. insanus*, but these species differed in the T6 cluster with *D. bicolor* having approximately 12 more T6 glomeruli than *D. insanus* (**Figure 4B**). Conversely, *F. mccooki*, which showed estimated total *s. basiconica* numbers comparable to *D. insanus* (**Figure 3D**), had nearly the same number of T6 glomeruli and the difference in glomeruli numbers between these species was in non-T6 glomeruli (**Figure 4B**). Thus, estimated total *s. basiconica* number corresponded best with the number of glomeruli in the T6 cluster.

Potential Pheromone Compounds

A total of 58 peaks were isolated through GC-MS for both *Dorymyrmex* species and all body parts analyzed (**Supplementary Tables 4, 5** and **Supplementary Figure 5**), including a total of 43 peaks from *D. bicolor* and 44 from *D. insanus* (**Supplementary Table 6**). This analysis identified a number of compounds known to be recruitment or alarm pheromone components in other dolichoderines, including the monoterpenoids iridomyrmecin, citronellal (3,7-dimethyloct-6-en-1-al), and limonene, and the ketone sulcatone (6-methyl-5-heptenone) (**Supplementary Figure 5**; Cavill and Ford, 1953; Cavill and Hinterberger, 1960; Blum et al., 1963; Crewe and Blum, 1971; Cavill et al., 1979; Tomalski et al., 1987). A large number of the peaks were identified as unbranched alkanes, with the majority isolated from both metasoma ("gaster") (G) and combined head and mesosoma ("thorax"; HT) samples (**Supplementary Table 5** and **Supplementary Figure 5**), suggesting they are distributed across the body. The total number of shared and unique compounds was comparable between the species, with 14 compounds unique to *D. bicolor* and 15 unique to *D. insanus* (**Supplementary Table 6**). This analysis detected a set of peaks between 16.07 and 17.02 min unique to *D. insanus* and a set of peaks between 18.61 and 19.12 min unique to *D. bicolor*, identified as acyclic alkanes. Nearly one third (17 of 58) peaks did not match existing records in the NIST library, and 13 of 58 were identified as having multiple matches (**Supplementary Table 5**).

Behavior

We measured interactions in 11 nestmate and 18 non-nestmate pairs of *D. bicolor* and in 11 nestmate and 16 non-nestmate pairs of *D. insanus* (**Figure 5**). Overall, *D. bicolor* pairs interacted more with each other than *D. insanus*, shown by more frequent incidental or fast contact (**Figure 5C**). While *D. bicolor* non-nestmate pairs had a greater number of aggressive interactions (**Figure 5D**) and significantly fewer affiliative interactions than nestmates (**Figure 5E**), *D. insanus* pairs showed no significant differences in these interactions (**Figures 5D,E**). We also recorded trophallaxis, an important means of sharing resources among members of a nest, and we found that *D. bicolor* nestmate pairs engage in this behavior more frequently than non-nestmates (**Figure 5H**). We recorded very few trophallaxis events for *D. insanus* and there were no differences in trophallaxis frequency between nestmates and non-nestmates (**Figure 5H**). Since differences in contact could be explained by differences in general activity or movement, and since we are specifically interested in sensory structures on the antennae, we also looked at the time pairs spent engaged in antennation. In these comparisons only pairs that showed this behavior were included in the analysis. A greater percentage of D. bicolor pairs showed this behavior (25 out of 29 pairs of *D. bicolor* and 13 out of 27 pairs of *D. insanus*; $p = 0.0038$, Fisher's exact test). We found that *D. bicolor* nestmates spend a longer total time antennating than non-nestmates, with the duration of antennation bouts being longer between nestmates (**Figures 5F,G**). We did not detect differences in total antennation time or bout duration between *D. insanus* nestmates and non-nestmates (**Figures 5F,G**).

DISCUSSION

In the experiments described here we tested whether differences in sensory and antennal lobe structures across workers of three species of odorous ants (Formicidae, Dolichoderinae) are explained by body size scaling or variation in colony size. We hypothesized that variation in visual sensory systems would be explained largely by body size and that differences due to colony size, if they exist, would be found in the olfactory system structures implicated in social communication, specifically nestmate recognition, in ants. We find that body size explains most of the variation in sensory structures across our sample of dolichoderine ants. Olfactory structures in general vary with body size and not with colony size across all species, but components of the olfactory system related to social cue processing, specifically *sensilla basiconica* and T6 glomeruli, do not follow patterns of body size variation seen for other sensory structures. Instead, the small-bodied *F. mccooki* had significantly fewer *s. basiconica* than the large-bodied *D. bicolor* but not the medium-sized *D. insanus*. This suggests either the small-colony *D. insanus* has fewer *s. basiconica* than would be predicted from body size, or its larger colony relative, *D. bicolor* has far more. This class of sensilla is associated with nestmate recognition in ants (Ozaki et al., 2005) and innervates the T6 cluster of glomeruli in the antennal lobe (Kelber et al., 2010). Observed differences in total glomeruli number among *D. bicolor*, *D. insanus*, and *F. mccooki* are explained by differences in the T6 cluster, suggesting differences between the *Dormyrmex* species are due to greater investment in these structures in *D. bicolor*. In probing functional consequences of this difference, we found *D. bicolor* show a greater number of social interactions than *D. insanus*, contacting both nestmates and non-nestmates more frequently, and displaying more affiliative and aggressive behaviors, respectively, during these pairings. Without clear taxonomic classifications and a robust phylogeny for *Dorymyrmex*, it is difficult to assess whether these traits are expanded in *D. bicolor* or reduced in *D. insanus*, but our findings suggest a link between colony size and these sensory structures.

Body Size and Sensory Structure Scaling

Intraspecific differences in body size are associated with variation in behavior in Hymenoptera (Nowbahari et al., 1999; Spaethe and Weidenmüller, 2002), including division of labor in ants (Wilson, 1980; Muscedere and Traniello, 2012), and sensory structure scaling (Renthal et al., 2003; Smallegange et al., 2008; Kelber et al., 2010; Perl and Niven, 2016). Sensilla density determines behavioral sensitivity for some tasks (Gill et al., 2013; but see Leitner et al., 2019). Interestingly, the number of olfactory glomeruli can vary across morphological castes in ants (Mysore et al., 2009; Kuebler et al., 2010), suggesting the regulation of olfactory receptor expression may be linked with body size within some species. Despite documented sensory structure scaling in solitary and social insects, behavioral consequences are not well-studied. In the buff-tailed bumblebee (*Bombus terrestris*) both sensilla number and odor sensitivity scale positively with body size intraspecifically (Spaethe et al., 2007), but little is known about functional consequences of interspecific scaling. Given that variation in individual sensory thresholds is hypothesized to underly division of labor in social insects (Beshers et al., 1999), regulation of worker size may be one mechanism to achieve this (Beshers and Fewell, 2001). Across closely related species, body size differences may influence differences in sensory structures and, by extension, sensory reception or perception.

In the ants studied here, sensory structure size and component number are positively correlated with body size across species. However, while the visual system generally scales with body size, *F. mccooki* eye area and ommatidia number do not scale with the same slope as *Dorymyrmex* spp. (**Figures 2G,H**), resulting in a significantly greater ommatidia density in this smaller species (**Supplementary Figure 3**). In our previous work we did not detect statistically significant differences in visual regions of the brains of these species, but *F. mccooki* trended toward greater mean investment in visual regions than would be predicted based on body or brain size rankings (Godfrey and Gronenberg, 2019b). It would be interesting to investigate differences in visually guided behavior across species, because while their foraging times in the Sonoran Desert do overlap, *F. mccooki* foraging times extend farther into the middle of the day than *Dorymyrmex* spp. (*personal observation*). Similar to visual systems, total antennal sensilla density (of all types, including *s. basiconica)* scales negatively with body size on both the penultimate and club segments (**Supplementary Figure 4B**), an effect driven by non-basiconic sensilla, since this holds true when basiconic sensilla are removed and non-basiconic sensilla are analyzed separately (**Figure 3B**).

We previously reported that antennal lobe size expressed as a relative proportion of brain size scales negatively with colony size in these ants (Godfrey and Gronenberg, 2019b), but here we find the total number of glomeruli can be ranked by body size across species (**Figure 4A**). In insects a one-to-one correspondence between olfactory receptors and glomeruli has been observed (Vosshall et al., 2000; Robertson and Wanner, 2006, but see Fishilevich and Vosshall, 2005; Younger et al., 2020), and we did not have reason to expect the total number of glomeruli (as an estimate of olfactory receptors) to scale positively with body size as seen here. Intraspecific variation in glomeruli number based on body size has been reported in the polymorphic ants *Camponotus compressa* (subfamily Formicinae) and *Atta wollenweideri* (subfamily Myrmicinae). In *A. wollenweideri,* smaller workers have fewer sensilla and antennal lobe glomeruli, whereas these numbers scale negatively with body size in *C. compressa* such that the smallest class of workers have the most sensilla and glomeruli (Mysore et al., 2009, 2010). Thus, intraspecific variation may be due to differences in gene expression linked to body size (Mysore et al., 2010), and, if so, in dramatically different ways across species. It is possible that similar mechanisms linking body size with gene expression could drive odorant receptor differences coincident with body size across very closely related species.

Evidence for Colony Size-Driven Differences in Sensory Structures

There exists little information on sensory trait evolution and social complexity, though studies generally predict a decrease in sensory system component number or size with transitions to sociality or increases in colony size. Indeed, in halictid bees, reductions in total sensilla density coincide with transitions from social to solitary habits, suggesting that even broad patterns of sensory investment may be related to social structure (Wittwer et al., 2017). While nearly all of the sensory structure variables in this study could be correlated with body size, *s. basiconica* density specifically could not be ranked by body size. However, this variable could also not be neatly linked with colony size across all three species. Gronenberg and Riveros (2009) suggested brain trait scaling may form a humped-shaped curve in relation to social complexity such that on the extreme ends there exists lower investment in structures involved in complex behaviors or social signaling, and Riveros et al. (2012) find support for this across olfactory structures in fungus-growing ants, but it is difficult to assess this with only the three species. Instead, our data indicate that *D. bicolor* may invest proportionally more in *s. basiconica* than its close relative, the smaller-colony *D. insanus*, resulting in a greater number of these socially relevant sensilla in the large-colony species. Total glomeruli counts show *D. bicolor* has 15–16 more total antennal lobe glomeruli than *D. insanus* (**Figure 4A**) and, given that olfactory sensory neurons (OSNs) housed in *s. basiconica* form the presynaptic terminals of the T6 glomeruli (Kuebler et al., 2010), we hypothesized that differences in the total number of glomeruli would be explained by differences in this cluster. Indeed, the T6 cluster differed by an average of 12 glomeruli between species (**Figure 4B**, *D. bicolor* $\bar{x} = 54$, $s = 7.4$ vs. *D. insanus,*$\bar{x} = 42$, $s = 2.1$), indicating the difference in the antennal lobe may be linked to differences in total number of *s. basiconica.* This kind of expansion in sensory reception systems has been attributed to strong selection for systems that maintain group cohesion and efficiently coordinate group behaviors (Leonhardt et al., 2016).

Because the OSNs of *s. basiconica* respond to large, cuticular hydrocarbons that act as nestmate recognition cues in ants (Ozaki et al., 2005), we predicted *D. bicolor* might show a greater diversity or a higher number of these compounds than *D. insanus*. A preliminary analysis of compounds found on the body and inside the gaster of *D. bicolor* and *D. insanus* revealed no differences that correspond clearly with colony size or sensory structure scaling. While analysis showed a set of large, unbranched alkanes unique to *D. bicolor* (**Supplementary Table 5** and **Supplementary Figure 5**; peaks 44, 49, 51, 53, 54, 55), there was a set similar in number unique to *D. insanus* (**Supplementary Table 5** and **Supplementary Figure 5**; peaks 37–40, 41). In many ant species, Dufour's gland produces alkanes used as recruitment and defensive signals (Cavill and Ford, 1953; Lenoir et al., 2011), but the broad distribution reported here suggests at least some of these may be cuticular hydrocarbons (**Supplementary Table 5**). However, cuticular hydrocarbon-based signaling among conspecifics in social species may not actually involve more varied or complex signaling molecules when compared with signaling in solitary species (Kather and Martin, 2015), nor is there evidence for a relationship between social complexity and signal complexity across ants (Ord and Garcia-Porta, 2012). However, the morphology required to interpret specific compounds may be expanded in social species (Dacks and Nighorn, 2011; Zhou et al., 2015). This is in line with our observation of expanded *s. basiconica* and T6 glomeruli in *D. bicolor,* when compared with *D. insanus,* without detectable qualitative differences in compounds produced by these species.

Colony size may be causally related to cuticular hydrocarbon profiles through genetic diversity. Polygynous colonies are often larger (Buczkowski and Bennett, 2008; dos Reis et al., 2011; Boulay et al., 2014) and the genetic diversity associated with polygyny may result in greater CHC diversity or variation in olfactory receptors, though evidence from the ant *Formica exsecta* suggests that polygyny may actually reduce CHC diversity (Martin et al., 2009). It is possible that the differences between *D. bicolor* and *D. insanus* stem not only from colony size directly, but from polygyny and associated differences in colony-level genetic diversity. However, while Nickerson et al. (1975) suggest both polygynous and monogynous species of *Dorymyrmex* exist in North America, queen number is not known for species used in this study.

In a previous study we found differences in exploratory behavior linked with colony size in these species, with the smaller-colony *D. insanus* showing greater exploratory activity. From those experiments we suggested these differences may be driven in part by differences in social interactions such as extended periods of contact in *D. bicolor* (Godfrey and Gronenberg, 2019b). Here, the differences in socially relevant sensory systems between *D. bicolor* and *D. insanus* correlate with the frequency and duration of social interaction within these species, suggesting observed differences in *s. basiconica* and T6 glomeruli may indeed be functional.

Undoubtedly, colony size plays a role in social insect evolution, as it is involved in both proximate (e.g., Jeanson et al., 2007; Ferguson-Gow et al., 2014) and ultimate (e.g., Robinson and Page, 1988) causes of worker trait variation or specialization. The current study focuses on the relationship between colony size and the size and number of sensory system components, with an emphasis on differences in sensory structures known to be involved in nestmate recognition, though not exclusively used for that purpose (D'Ettorre et al., 2017). We find evidence that communication systems change with colony size in closely related species, potentially because selection acts on nestmate recognition and signaling related to cooperative behaviors. However, our data do not support a universal pattern of sensory system scaling associated with changes in colony size. Here we compare a small number of species for which a clear taxonomy and robust phylogeny are only now being assembled, so we offer no *a priori* hypothesis regarding causation or directional evolution; it seems equally possible that shifts in communication systems drove expansion of colony size (LeBoeuf et al., 2013) as the inverse (Riveros et al., 2012), and these traits very likely coevolve.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: University of Arizona Research Data Repository (ReData) 10.25422/azu.data.14878110.

AUTHOR CONTRIBUTIONS

RKG conceived, designed the experiments, collected, analyzed the data, and wrote the manuscript. JTO provided taxonomic assessment of species and created figures. TA, CG, JH-R, and JTO collected, analyzed the data, and provided critical revisions of the manuscript. WG assisted in experimental design, created figures, and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.733023/full#supplementary-material

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Behavioral Attributes of Social Groups Determine the Strength and Direction of Selection on Neural Investment

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The evolution of social systems can place novel selective forces on investment in expensive neural tissue by changing cognitive demands. Previous hypotheses about the impact of sociality on neural investment have received equivocal support when tested across diverse taxonomic groups and social structures. We suggest previous models for social behavior-brain relationships have overlooked important variation in social groups. Social groups vary significantly in structure and function, and the specific attributes of a social group may be more relevant to setting cognitive demands than sociality in general. We have identified intragroup competition, relationship differentiation, information sharing, dominance hierarchies, and task specialization and redundancy as attributes of social behavior which may impact selection for neural investment, and outline how variation in these attributes can result in increased or decreased neural investment with transitions to sociality in different taxa. Finally, we test some of the predictions generated using this framework in a phylogenetic comparison of neural tissue investment in Anelosimus social spiders. Social Anelosimus spiders engage in cooperative prey capture and brood care, which allows for individual redundancy in the completion of these tasks. We hypothesized that in social spider species, the presence of redundancy would reduce selection for individual neural investment relative to subsocial species. We found that social species had significantly decreased investment in the arcuate body, the cognitive center of the spider brain, supporting our predictions. Future comparative tests of brain evolution in social species should account for the special behavioral characteristics that accompany social groups in the subject taxa.

Keywords: neuroecology, social behavior, social brain evolution, social spiders, distributed cognition hypothesis

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INTRODUCTION

Evolutionary transitions of social behavior can introduce novel cognitive demands (Silk, 2007). Metabolic costs of neural tissue are disproportionately high and brain tissue investment should be constrained to meet cognitive demands (Niven and Laughlin, 2008; Iwaniuk, 2017). Origins or modification of sociality may drive changes in brain tissue investment. Several hypotheses have been advanced to explain changes in neural tissue investment associated with transitions in social behavior, generally referred to as "social brain" hypotheses (Dunbar, 1998). Initially, social brain hypotheses assumed there would be a positive effect of sociality on brain investment, but more recent theoretical treatments (Gronenberg and Riveros, 2009; Lihoreau et al., 2012) and empirical findings (vertebrates: Gonzalez-Voyer et al., 2009; Fedorova et al., 2017; Kverková et al., 2018; social insects: O'Donnell et al., 2015) have called this assumption into question.

Studies to date have not adequately identified or accounted for varying behavioral selective pressures that could change cognitive demands as sociality evolves. Analyzing the diversity of social interactions can contribute to more rigorous theory linking social environments with brain evolution. We suggest comparative tests of social brain hypotheses should explicitly consider which aspects of behavior change during transitions from solitary to group living, or during transitions in social complexity, in each study taxon. We identify some key features of social systems that can act as independent selective forces on neural investment. We explore how each of the following attributes of social systems can affect cognitive demands and thus selection for neural investment: intragroup competition, individual recognition, information sharing, dominance hierarchies, and task performance specialization and redundancy. We predict that some of these behavioral attributes will decrease the fitness effects of individual cognitive abilities, such that selection for neural investment will be relaxed. In contrast, other attributes of sociality may place greater cognitive demands on individuals, selecting for increased neural investment.

It is important to note that few, if any, of the behavioral attributes we explore are universally present among social taxa. The relevance of these behavioral attributes vary among animal taxa, depending in part on how social groups form and function. An important complicating factor is the role of individual vs. group/colony selection as the main determinant of fitness in a species (O'Donnell et al., 2018). Variation among animal lineages in the structure of social groups provides opportunities for cross-taxon comparative analyses, which can be used to assess the relative importance of different behavioral factors as determinants of neural investment.

Our paper is divided into two main sections. In the first section we present some attributes of social behavior that could independently impact cognitive demands on animals, either increasing or relaxing selection for brain investment. We discuss potential effects of these demands on the evolution of brain investment. The second section is an empirical test of social brain effects in a taxon with diverse grades of sociality and a relatively simple social system (cobweb spiders: Avilés and Guevara, 2017). Social cobweb spider species exhibit task sharing and redundancy among females; social males and subsocial species do not (Samuk et al., 2011; Harwood and Avilés, 2013, 2018). Furthermore, social spiders do not exhibit some potentially confounding elements of sociality, such as dominance hierarchies and task specialization (Lubin, 1995; Settepani et al., 2013) that could complicate the interpretation of social brain effects comparisons.

ATTRIBUTES OF SOCIAL BEHAVIOR: INTRAGROUP COMPETITION

The evolution of social behavior potentially increases the frequency or intensity of competition between animals, and social competitive costs must nearly always be compensated by other fitness payoffs to favor sociality (Silk, 2007). Social group participation can drive increased brain tissue investment, either in response to or in anticipation of competitive interactions. Greater neural investment can improve performance on behavioral tasks, such as mate location, and may confer individual benefits in highly competitive social environments (Kotrschal et al., 2015). Potential relationships of heightened competition with brain investment are illustrated by species where social groups involve temporary or facultative aggregations that can increase local competition for food or other resources. In desert locusts, the transition from solitary living to the formation of massive swarms that compete for food and engage in cannibalism (Guttal et al., 2012) is accompanied by increased total brain investment and greater relative investment in the mushroom bodies, which are cognitive integrating regions of the insect brain (Ott and Rogers, 2010). Experimental manipulations that induce sociality in species that are typically solitary have found similar increases in cognitive performance or neural investment in social groups. In *Drosophila melanogaster* fruit flies, larval crowding resulted in increased food competition and was associated with greater adult neural investment (Heisenberg et al., 1995); juvenile *Marpissa muscosa* jumping spiders reared in groups had significantly improved performance on learning assays although competition was not directly implicated in this study (Liedtke and Schneider, 2017).

ATTRIBUTES OF SOCIAL BEHAVIOR: INDIVIDUAL RECOGNITION

Individual recognition can be cognitively demanding because it requires discriminating and remembering the individual identities of social partners (Shultz and Dunbar, 2006; Bergman and Beehner, 2015). In addition to the number of unique relationships, qualitative differences in interactions between group members can impose different cognitive demands. For example, monogamous pair bonds are common among birds, but species vary in the level of pairs' cooperation and affiliative behaviors (Emery et al., 2007). Emery et al. (2007) proposed that increased total brain investment in some bird species can be attributed to a higher degree of cooperation during brood care, increased affiliative displays, and social support interactions among mates. Social alliances in particular may be expected to impose higher cognitive demands because of the necessity to remember group member identities, predict the outcome of interactions, and recognize others with similar status (Chapais, 1995).

ATTRIBUTES OF SOCIAL BEHAVIOR: DOMINANCE HIERARCHIES

Dominance hierarchies describe ranks of group members in relation to one another (Drews, 1993). The criteria used to define a "dominant position" vary among species but may include increased access to food resources or reproductive opportunities relative to other members of a group (Drews, 1993). Achieving dominant status and maintaining a hierarchy may increase cognitive demands (and brain investment) by requiring dominant individuals to differentiate group member relationships (Dunbar, 1992). Socially dominant positions in hierarchies are associated with increased neural investment in socially flexible bees relative to both solitary and subordinate individuals (Smith et al., 2010; Rehan et al., 2015). A loss of sociality in a primitively social bee lineage resulted in decreased foundress neural investment, potentially due to the loss of dominance interactions (Pahlke et al., 2019, 2020).

The expected relationship of dominance behavior with brain investment is complicated because dominance hierarchies do not always rely on cognitively demanding, individualized aggressive behaviors. Some hierarchies rely partially or entirely on chemical signaling (Faulkes and Bennett, 2001; Sundström and Boomsma, 2001; Orlova et al., 2020). In hierarchies which do not rely on aggressive interactions, selection for neural investment to navigate social interactions may be minimal (Lihoreau et al., 2012). An additional complication is that dominance status can affect other aspects of behavior such as task performance. For example, reproductively-dominant social insect queens may perform few behaviors other than egg laying (Noirot, 1989; Barchuk et al., 2018), potentially permitting reduced brain investment for dominants compared to subordinates (Barchuk et al., 2018; Gordon et al., 2019; but see O'Donnell et al., 2017). In these systems, neural investment in dominants and subordinates may no longer be driven by social interactions, but instead by caste-specific division of labor (thus falling under the task specialization attribute, described below).

ATTRIBUTES OF SOCIAL BEHAVIOR: INFORMATION SHARING

Information sharing allows social animals to respond adaptively to stimuli they have not experienced directly. Information sharing can decrease the fitness costs of inaccurate stimulus detection and may reduce selection for individual cognitive abilities, permitting decreased brain tissue investment. The effect of group information sharing on cognitive demands is well-illustrated by vigilance behaviors. Individual vigilance behavior performance rates decrease in the presence of conspecifics in some species (McBlain et al., 2020; Wang et al., 2021), and predator detection can be faster and more accurate in social groups compared to lone individuals (Ward et al., 2011). However, the need for increased ability to produce, detect and respond to social signals can involve additional cognitive and neural costs. In this case, social and environmental information may be integrated by different parts of the brain, resulting in distinct patterns of mosaic brain evolution: in cichlid fish, environmental complexity is associated with increased telencephalon investment, while increased social complexity is associated with increased hypothalamus investment (Pollen et al., 2007). Thus, for systems in which information sharing occurs, assessing changes in specific brain regions, rather than total brain investment, may better detect the effects of changes in cognitive demands on brain investment.

ATTRIBUTES OF SOCIAL BEHAVIOR: TASK PERFORMANCE SPECIALIZATION AND REDUNDANCY

A common theme in some taxa is for social group members to specialize on a subset of the tasks typically performed by solitary-living individuals (Jeanne, 1986). Individuals may specialize on specific behaviors for all or part of their lifetime; in extreme cases, the evolution of morphologically distinct castes in some social insects limits workers to performing certain behaviors or tasks (Korb and Thorne, 2017; O'Donnell et al., 2018; Gordon et al., 2019). Task specialization is often paired with neural adaptations to meet the cognitive demands of a specific behavioral class (Amador-Vargas et al., 2015; Iwaniuk, 2017), but may result in decreased selection for general cognition (Gronenberg and Riveros, 2009). Task specialization may improve colony-level efficiency (Keller et al., 2011), and decreases in individual neural investment can represent a savings of resource investment by the social group. Reduced individual neural investment may mark a transition to colony-level selection on neural investment (O'Donnell et al., 2015, 2018).

Redundancy, or the availability of multiple individuals to perform or complete a given task, may also reduce individual cognitive demands. Redundancy can facilitate high probabilities of successful task completion even when individual competency is relatively low (Herbers, 1981). Redundancy in colony labor is often present in caste-specialist systems and may be critical for effective task partitioning among group mates (Jeanne, 1999), but redundancy is not restricted to task-specialist systems. Redundancy applies whenever the completion of a shared task is not the responsibility of a single individual, such as in cooperatively breeding social groups (Taborsky et al., 2007; Riehl, 2013). Decreased neural investment has been associated with living in stable groups in woodpeckers (Fedorova et al., 2017), and in cichlid fishes who engage in biparental care (Gonzalez-Voyer et al., 2009). In both of these systems redundancy to complete tasks may reduce individual cognitive demands. In fact, the evolution of redundant cooperation may be more likely to occur when individuals are unaware of group-mates' social strategies (Pedroso, 2020).

SOCIAL SPIDERS: A TEST FOR SOCIAL BRAIN EFFECTS IN A SIMPLE SOCIAL SYSTEM

Several evolutionary transitions between subsocial and social behavior have occurred in the cobweb spider genus *Anelosimus* (**Figure 1**). In subsocial species, individual females perform extended maternal care but offspring disperse prior to or at adulthood (Yip and Rayor, 2014; Avilés and Guevara, 2017). In social species the offspring do not disperse, leading to the maintenance of permanently communal nests. Social species adult females cooperatively capture prey and share offspring care (Avilés and Tufino, 1998; Yip et al., 2008; Samuk and Avilés, 2013; Avilés and Guevara, 2017). However, social *Anelosimus* spiders lack task specialization and do not exhibit individual recognition or dominance hierarchies (Jones and Parker, 2000; Aviles and Bukowski, 2006; Settepani et al., 2013). Individual redundancy, resulting from cooperation on maternal care and prey capture, could drive reduced selection for individual competency at these tasks. As predicted, individual females in social *Anelosimus* species are less competent at maternal care (Samuk et al., 2011) and less effective hunters (Harwood and Avilés, 2018) when compared to females from related subsocial species. We expected brain investment to differ among the sociality levels due to differences in reliance on individual ability to successfully complete maternal care and prey capture tasks. We predicted the arcuate body would be less developed (relatively smaller) in females of the social compared to subsocial species, because the arcuate body is a center of mechanosensory integration (Babu and Barth, 1984; Steinhoff et al., 2018). Unlike females, males of both social and subsocial species die shortly following mating (Avilés and Gelsey, 1998; Avilés and Salazar, 1999; Viera et al., 2007) and have little or no engagement in colony activity, except in nest maintenance (Aviles, 1986). Because male behavior does not differ consistently between the sociality categories, we predicted male brain investment patterns would be similar in subsocial and social species.

METHODS: A TEST FOR SOCIAL BRAIN EFFECTS IN A SIMPLE SOCIAL SYSTEM

Subject Spider Collections

Specimens were collected and preserved in Prefer aldehyde-based fixative solution (Anatech, Ltd.). We sampled adult females from 5 social and 6 subsocial species of *Anelosimus* spiders (adult males from 5 social and 4 subsocial species), representing at least 3 independent transitions to social behavior (Agnarsson et al., 2006; **Figure 1C**). **Subsocial species:** *A. arizona* (n = 3 females, 3 males); *A. baeza* (n = 4 females, 3 males); *A. elegans* (n = 3 females, 3 males); *A. jabaquara* (n = 3 females); *A. studiosus* (n = 3 females, 3 males); *A. tosum* (n = 2 females). **Social species:** *A. domingo* (n = 1 female, 3 males); *A. dubiosus* (n = 3 females, 1 male); *A. eximius* (n = 3 females, 3 males); *A. guacamayos* (n = 3 females, 3 males); *A. oritoyacu* (n = 3 females, 3 males). Specimen collection date and location are detailed in **Supplementary Material**.

Histology and Brain Size Measurements

For each subject we removed the legs and opisthosoma and opened the cephalothorax dorsum to remove the venom gland, then embedded in plastic resin comprised of Embed-812 resin,

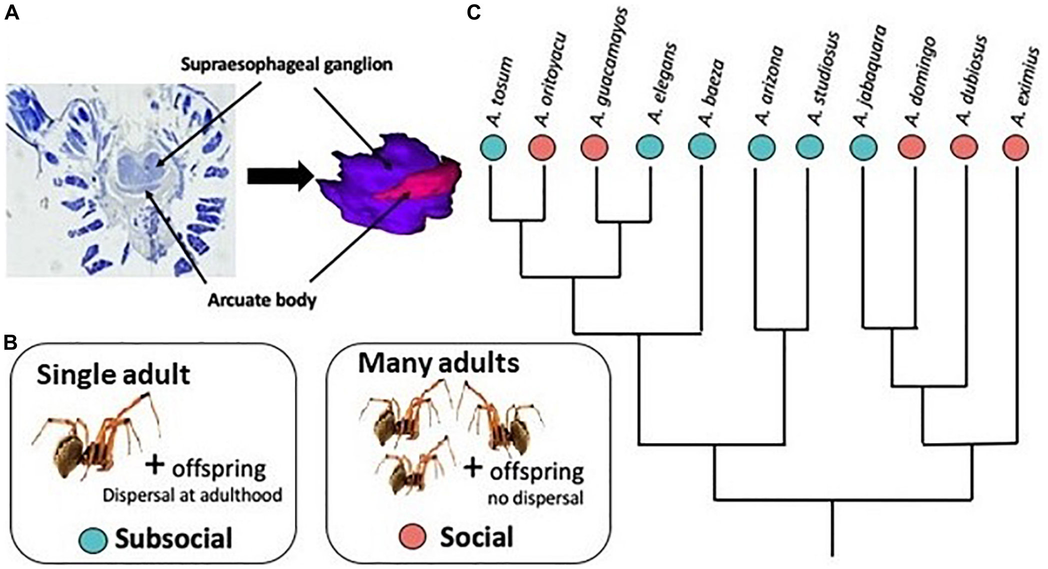

FIGURE 1 | (A) Light photomicrographs of stained and sectioned protocerebrum tissue, showing supraesophageal ganglion (SEG) and arcuate body (AB) regions. The 3D reconstruction shows the relative sizes of the SEG (purple) and AB (pink). **(B)** Brain volume was compared between subsocial (blue) species, which form nests of single mothers and their offspring, who disperse at adulthood, and social (pink) species, which form permanent communal nests with multiple mothers and offspring. **(C)** Phylogenetic relationships among the sampled species (Luo et al., 2020).

DDSA, DBP, and DMP 30 (Electron Microscopy Services, Inc.), and cured at 60°C for 72 h. Embedded tissue was sectioned into 8–10 um thick slices using a rotary microtome and stained using Toluidine Blue O (Fisher Scientific). Sections were photographed using a light microscope and areas containing neuropil were outlined using ImageJ version 1.53a (Schneider et al., 2012), multiplied by slice thickness, and then converted to volume (mm^3) using a pixel-to-millimeter conversion factor. Detailed embedding protocol (O'Donnell et al., 2011).

For each specimen, we measured the protocerebrum, which is considered to be the "true brain" of spiders and is comprised of the supraesophageal ganglion and the arcuate body (Babu and Barth, 1984). The supraesophageal ganglion receives visual sensory input (Babu and Barth, 1984). The arcuate body is an integration center between the subesophageal and supraesophageal ganglia (Babu and Barth, 1984), and may be important for navigating complex environments (Steinhoff et al., 2018). We focused on the arcuate body as a region particular relevant to maternal care tasks and prey capture, due to the necessity to integrate visual and mechanosensory information and coordinate locomotion during these tasks (Steinhoff et al., 2018). We quantified supraesophageal volume (as a brain size reference) and arcuate body volume (Steinhoff et al., 2017).

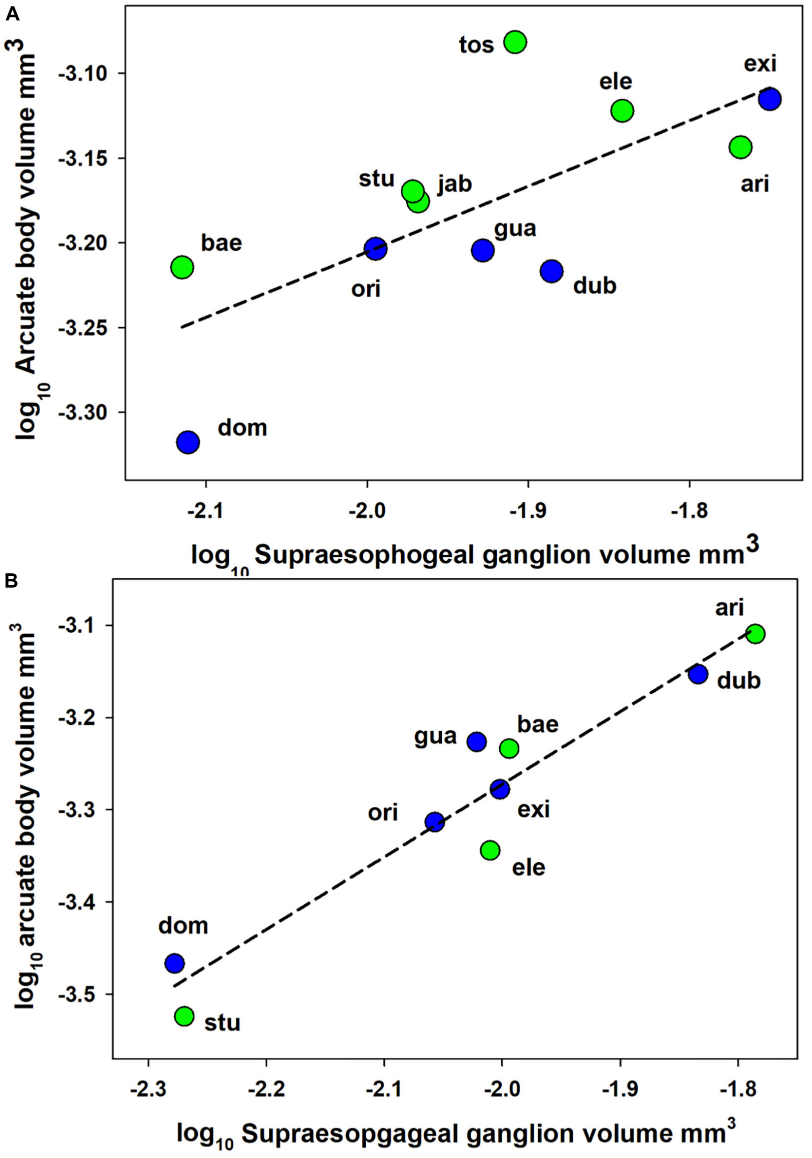

FIGURE 2 | Relationships between log supraesophageal ganglion volume and log arcuate body volume for *Anelosimus* spiders. **(A)** Females. **(B)** Males. Data points represent mean-species values for either social (blue) or subsocial (green) species. Lines represents slopes of linear regression calculated using data from all species. ari *A, Arizona*; bae *A, baeza*; dom *A, domingo;* dub *A, dubiosus*; ele *A, elegans*; exi *A, eximius*; gua *A, guacamayos*; jab *A, jabaquara*; ori *A,oritoyacu*; stu *A, studiosus*; tos *A, tosum*.

Statistical Analysis

We used General Linear Models (GLM; SPSS v. 28) and Phylogenetically Generalized Least Squares (PGLS; R version 4.0.2 and the packages ape (Paradis and Schliep, 2019) and phytools (Revell, 2012) to test whether subsocial and social species differed in relative arcuate body investment. We performed separate analyses for male and female brain volume data. Because we expected an allometric relationship between arcuate body volume and brain size (Napiórkowska and Kobak, 2018), we first accounted for brain size effects using supraesophageal ganglion volume as a covariate in the models, then tested for the effects of species sociality category (subsocial vs. social) as a fixed factor. We performed GLM analyses (with type I sums of squares) on the raw species-mean brain data and on log10-transformed brain region volumes to assess the allometry of brain region evolution (Mascaro et al., 2014). To account for possible effects of phylogeny on brain region/behavior relationships we also performed separate phylogenetically generalized least squares regression (PGLS). PGLS allows accounting for linear covariates in the analysis of categorical differences in response variables (in our study, social behavior category differences in arcuate body volume) (Mundry, 2014). We used a published phylogeny of *Anelosimus* species from Luo et al. (2020), with all branch lengths set to one. We used a PGLS to test the relationship between arcuate body investment and sociality level, with supraesophageal ganglion volume as a covariate (controlling for brain size, as we did in the GLM). Mean species values of brain region volumes used in PGLS analysis were log10 transformed.

RESULTS: A TEST FOR SOCIAL BRAIN EFFECTS IN A SIMPLE SOCIAL SYSTEM

For females, social category was a significant predictor of arcuate body volume. After accounting for significant effects of brain size [supraesophageal ganglion volume: GLM $F_{(1,\ 8)} = 12.02$, $p = 0.008$], arcuate body volume was significantly greater in subsocial than in social species [GLM $F_{(1,\ 8)} = 5.72$, $p = 0.04$]. Similar patterns were found in the analysis of log-log transformed brain volume data [**Figure 2**; supraesophageal ganglion volume effect: GLM $F_{(1,\ 8)} = 16.15$, $p = 0.004$; social category effect: GLM $F_{(1\ ,8)} = 7.03$, $p = 0.03$]. The log-log slope of the arcurate body vs. supraesophageal ganglion volume regression was significantly lower than 1.0 (slope = 0.39 ± 0.24 95% CI), demonstrated an allometrically decelerating rate of increase in arcuate body size. Using the PGLS to account for phylogeny supported the effects of sociality category: arcuate body size was greater in subsocial than in social species [**Figure 2**; PGLS on log transformed data: $F_{(1,\ 8)} = 14.84$, $p = 0.005$]. For males, social behavior category did not predict arcuate body size [GLM linear analysis $F_{(1,\ 6)} = 1.11$, $p = 0.33$; GLM log-log analysis $F_{(1,\ 6)} = 1.72$, $p = 0.24$; PGLS on log transformed data $F_{(1,\ 6)} = 3.3$, $p = 0.12$].

DISCUSSION: A TEST FOR SOCIAL BRAIN EFFECTS IN A SIMPLE SOCIAL SYSTEM

Female *Anelosimus* of social species engage in cooperative brood care and prey capture (Avilés and Tufino, 1998; Yip et al., 2008; Harwood and Avilés, 2013, 2018; Samuk and Avilés, 2013; Avilés and Guevara, 2017), and we predicted that individuals' redundancy in these tasks would reduce individual cognitive demands and relax selection for neural investment compared to subsocial congeners. As predicted, females of social species had significantly reduced relative investment in the arcuate body compared to subsocial species. This pattern did not hold for males which do not differ in task redundancy between subsocial and social species. These results indicate that behavioral redundancy is an attribute of sociality which may promote reduced brain region investment, independently of other social attributes such as task specialization. Application of the behavioral framework we develop to other social systems may encourage the identification of behavioral attributes driving brain evolution that have previously gone unnoticed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LA collected and supplied specimens. All authors listed have made substantial contributions to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.733228/full#supplementary-material

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REVIEW
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Slow-Fast Cognitive Phenotypes and Their Significance for Social Behavior: What Can We Learn From Honeybees?

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Cognitive variation is proposed to be the fundamental underlying factor that drives behavioral variation, yet it is still to be fully integrated with the observed variation at other phenotypic levels that has recently been unified under the common pace-of-life framework. This cognitive and the resulting behavioral diversity is especially significant in the context of a social group, the performance of which is a collective outcome of this diversity. In this review, we argue about the utility of classifying cognitive traits along a slow-fast continuum in the larger context of the pace-of-life framework. Using Tinbergen's explanatory framework for different levels of analyses and drawing from the large body of knowledge about honeybee behavior, we discuss the observed interindividual variation in cognitive traits and slow-fast cognitive phenotypes from an adaptive, evolutionary, mechanistic and developmental perspective. We discuss the challenges in this endeavor and suggest possible next steps in terms of methodological, statistical and theoretical approaches to move the field forward for an integrative understanding of how slow-fast cognitive differences, by influencing collective behavior, impact social evolution.

Keywords: cognitive phenotypes, pace of life, speed-accuracy tradeoff, social behavior, honeybees

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INTRODUCTION

In the classic story of the tortoise and the hare, we learn about two distinctly different personalities and the lesson that speed does not always matter, and an individual can be as successful doing things slowly. Understanding questions regarding phenotypic variations such as why some animals are slow and others are fast is a fundamental question in biology that has a long history in terms of r and k selection and life history theory (MacArthur and Wilson, 1967; Pianka, 1970; Stearns, 1976). Such slow-fast differences in behavior, both between and within a species, have been modeled under the pace-of-life syndrome (POLS) framework (Ricklefs and Wikelski, 2002; Réale et al., 2007, 2010), which proposes a suite of contrasting phenotypic traits to characterize this slow-fast axis (**Figure 1**). In this framework, a slow pace-of-life, characterized by life history traits such as slow growth, delayed reproduction and high survival, and a fast pace-of-life, marked by fast growth, early reproduction and low survival, are functionally mediated by a large set of correlated physiological and behavioral traits.

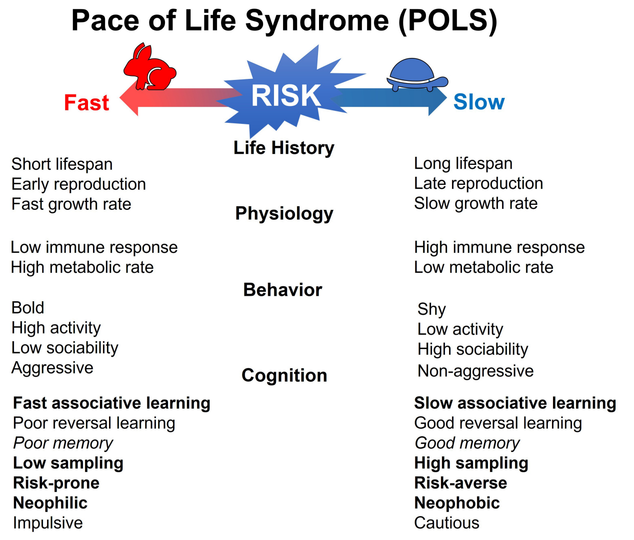

FIGURE 1 | A summary of the predicted integration between the slow-fast pace-of-life axis at different phenotypic levels (life-history, physiology and behavior) and the slow-fast cognitive axis defined by the speed-accuracy trade-off, with examples of a few traits at each level (after Réale et al., 2010; Sih and Del Giudice, 2012). Specifically with respective to cognition, traits in bold letters are those for which the empirical data met theoretical predictions, those in italics did not meet predictions, and the remaining are those that were not tested (Tait and Naug, 2020).

Behavioral variation correlates to slow-fast life history differences through a risk-reward trade-off in which the higher expression of certain behaviors can bring more rewards, but at the cost of higher risk (Stamps, 2007; Wolf et al., 2007). Behaviorally, fast individuals are those who can engage in more risk-taking behaviors that allow them to gather resources more rapidly and thereby express the traits associated with a faster life history, compared to slow individuals. Since behavioral output is an outcome of underlying cognitive mechanisms, these slow-fast behavioral differences are proposed to be outcomes of a speed-accuracy trade-off in terms of decision-making (Chittka et al., 2009; Sih and Del Giudice, 2012; Jolles et al., 2020). Fast decisions made with little information are subject to higher inaccuracy but can result in greater and more immediate rewards while slower decisions made with more information are predicted to improve accuracy but come at the cost of immediacy. The cognitive axis resulting from this trade-off predicts fast individuals to broadly demonstrate rapid learning, lower sampling and poor retention of information, relative to slow individuals (**Figure 1**). Although a link between behavioral and cognitive variation has been demonstrated to some extent (Amy et al., 2012; Cole et al., 2012; Dougherty and Guillette, 2018; Tait and Naug, 2020), most of these studies are still limited in their scope and examining the covariation of multiple cognitive traits with other phenotypic traits remains a significant challenge.

This recent interest in a covariance between cognitive and behavioral variation is mostly focused at the between-individual level within a species (Carere and Locurto, 2011; Thornton et al., 2014; Griffin et al., 2015; Boogert et al., 2018). In contrast, the consequences of this individual level covariance structure have rarely been studied at the level of a group, even though the pace-of-life framework provides a convenient basis to understand this interplay (Dammhahn et al., 2018). Group living provides an opportunity to potentially relax the constraints placed upon the individual by the tight correlation among a set of traits that define the trade-offs in terms of risks or rewards and speed or accuracy (**Figure 2**). This extends the idea that at the collective level, any phenotypic variation allows the collective phenotype to show a greater range of response to a variable, complex and multivariate environment (Piersma and Drent, 2003; Woods, 2014). However, the final expression of the collective phenotype can be more complex than predicted because the different phenotypes in a social group may modulate the performance of each other (Webster and Ward, 2011; van den Bos et al., 2013). Negative frequency dependent processes can increase the performance of a phenotype when it is rare while positive frequency dependent processes can decrease the performance of a rare phenotype.

For a comprehensive understanding of any biological question, it is important to take an integrative approach that encompasses both proximate (how?) and ultimate (why?) levels of analyses. In this review, we therefore use "Tinbergen's four questions" approach (Tinbergen, 1963) to address cognitive variation at four different levels—functional value, evolution, causation and development. Using this background, we argue that social insects, especially honeybees, provide ideal model systems to pursue integrative studies that span these different levels of inquiries at different levels of biological organization, using the pace-of-life as the central framework to understand the importance of slow-fast differences in cognition in the social context. We review our current understanding regarding cognitive variation in honeybees and identify existing gaps in knowledge, offering suggestions regarding potential methodological and statistical approaches that can help close these gaps.

INTERINDIVIDUAL VARIATION IN COGNITIVE TRAITS AND COGNITIVE PHENOTYPES IN SOCIAL INSECTS

Social insect colonies are comprised of significant morphological, physiological and behavioral variation among individuals, differences which underlie the observed division of labor that is widely considered to be at the heart of their extraordinary ecological success (Oster and Wilson, 1978; Beshers and Fewell, 2001). Although variation in cognitive traits is less studied, one of the best examples of such interindividual variation that is known to be correlated across multiple phenotypic levels comes from the extensive study of low and high strains of pollen hoarding honeybees (Pankiw and Page, 2000; Page et al., 2012). The two strains differ in their sensorimotor and learning abilities such that the high strain bees have

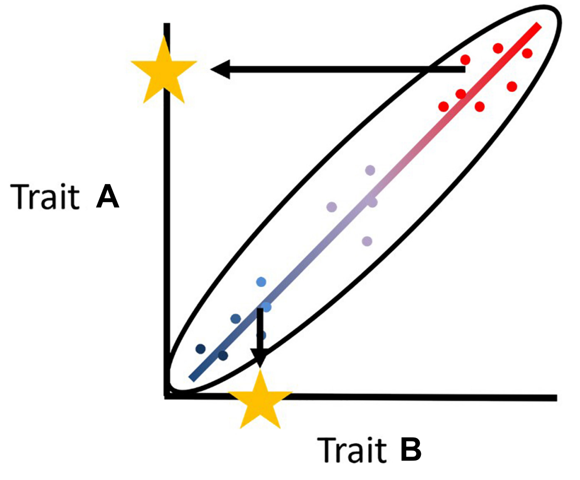

FIGURE 2 | Hypothetical correlation between two traits, **(A,B)** at the individual level that leads to possible slow (blue) and fast (red) phenotypes. This can lead to an individual being constrained in its phenotypic expression, defined by its tightly associated trait values for **(A,B)**. However, a group composed of both slow and fast phenotypes, by being able to express trait values at different points on the slow fast axis, is able to relax these constraints at the collective level.

higher sensitivity to sugar and water, a higher performance on odor- and tactile based associative learning tasks as well as an earlier age of first foraging, higher levels of vitellogenin and ovary development, a suite of traits referred to as the foraging syndrome (Page et al., 1998, 2006). The strong association among several learning traits and their correlation with other behavioral and life history traits are linked through a common genetic architecture of overlapping quantitative trait loci (QTLs) that show broad epistatic and pleiotropic effects (Page et al., 2012). These QTLs are also associated with insulin-insulin like signaling (IIS) and target of rapamycin (TOR) pathways, which have broad effects in nutritional signaling and regulation of behavior across a wide range of taxa, indicating the fundamental integration of cognition with a variety of traits at different levels of the phenotype. These observations, however, bring up the important question about the nature of causal relationships among these multitude of traits and it has been shown that differences in learning ability are largely explained by differences in sucrose sensitivity—a physiological trait (Scheiner et al., 2005; Roussel et al., 2009). Interindividual variation in cognitive traits has also been documented in other bees (Spaethe et al., 2007; Raine and Chittka, 2008; Muller and Chittka, 2012; Klein et al., 2017) and it is important to ask if this variation is similarly correlated to differences at other phenotypic levels as what is observed in the honeybees.

Although simple associative learning, due to the relative ease with which it can be measured, is the most commonly used trait to describe the cognitive phenotype of an individual, it is somewhat limited in its scope and one has to go beyond it if we are to understand the functional relevance of cognitive variation (Giurfa, 2015). Honeybees do show variation in other learning traits such as reversal learning (Bhagavan et al., 1994; Carr-Markell and Robinson, 2014; Cook et al., 2019) and aversive learning (Junca et al., 2019), and these different types of learning measures are correlated at the individual level such that there is a negative relationship between associative and reversal learning, and between appetitive and aversive learning abilities. Apart from these learning traits, little is known about similar variation with respect to other cognitive traits other than some limited work showing interindividual variability in terms of risk-sensitivity (Mayack and Naug, 2011), sampling and novelty preference (Katz and Naug, 2015), all of which are connected to energetic state. Using this as a background, it was more recently shown that several of these cognitive traits covary in a manner that largely meets the predictions of the speed-accuracy trade-off and results in slow and fast cognitive phenotypes such that fast bees are described by high associative learning and high preferences for novelty and risk, compared to slow bees (Tait and Naug, 2020). These cognitive differences also translated to functional differences in behavior and life history traits—fast bees transitioning to a forager role at an earlier age than slow bees. These findings suggest that slow-fast cognitive phenotypes describe broad differences among individuals in a variety of cognitive traits and provide a window into how cognitive variation fits into the pace-of-life framework.

There are two distinct foraging phenotypes in honeybees—scouts that gather new information about the resource environment and recruits which use that information (Seeley, 1983; Biesmeijer and De Vries, 2001). These two phenotypes can therefore be considered functionally equivalent to producers and scroungers (Katz and Naug, 2016), which allows us to consider how different cognitive phenotypes might occupy distinct social roles in a broader ecological framework (Katsnelson et al., 2011). The ability of producers to acquire information through personal experience should be reflected in their high performance on an individual learning task while the ability of scroungers to do the same by observing conspecifics should be reflected in their high performance on a social learning task. Given that individual learning should require higher levels of sampling (Kurvers et al., 2010; Rosa et al., 2012), one would predict that scouts represent the slow cognitive phenotype which spends more time gathering information. While this seems to be substantiated by the slower learning seen in scouts (Cook et al., 2019), it stands in contrast with their observed preference for novelty (Liang et al., 2012), which aligns more with what is expected of the fast cognitive phenotype (Sih and Del Giudice, 2012). Such inconsistencies highlight the fact that the original predictions of the slow-fast cognitive axis might not be as universal, an issue that we feel is also partly related to the challenge of clearly defining the cognitive traits themselves, an issue which we discuss later in more detail. In addition, the scout/recruit behavioral axis might also be plastic over the foraging lifespan of an individual bee, subject to its developmental, social and resource environment, which can lead to inconsistencies in the cognitive traits measured in these two phenotypes.

COGNITIVE DIVERSITY: ADAPTIVE VALUE OF SLOW-FAST COGNITIVE PHENOTYPES IN A GROUP

The historic and continued fascination with social insects is primarily to do with the fact that they are considered superorganisms due to their collective performance based on the tight integration among colony members (Wheeler, 1911; Wilson, 1971; Kennedy et al., 2017). This view of insect societies has been addressed in terms of several phenotypic traits (Lumsden, 1982; Southwick, 1983; Behmer, 2009; Gillooly et al., 2010) including cognition (Franks, 1989; Seeley, 1989; Couzin, 2009; Menzel, 2012; Feinerman and Korman, 2017). Phenotypic diversity among colony members (Jeanne, 1988), which allows flexibility and resilience to environmental heterogeneity and perturbations, promotes group productivity and fitness (Jones et al., 2004; Mattila and Seeley, 2007; Oldroyd and Fewell, 2007; Wray et al., 2011; Modlmeier et al., 2012) although there are studies which suggest that the effects of diversity could be more complex (Moritz and Page, 1999; Arathi and Spivak, 2001; Baer and Schmid-Hempel, 2001; Mugel and Naug, in press). Models of division of labor and task allocation addressing how interindividual behavioral variability affects group performance (Beshers and Fewell, 2001), have, however, included relatively little on the specific role of cognitive variation on collective performance. Even the recent interest in understanding the significance of interindividual variability within a social insect colony (Pinter-Wollman, 2012; Bengston and Jandt, 2014; Jandt et al., 2014; Jeanson and Weidenmuller, 2014; LeBoeuf and Grozinger, 2014; Jandt and Gordon, 2016) has not explicitly addressed the role of cognitive differences among individuals and its significance for social evolution.

The observed interindividual variation in cognitive traits at the colony level (Page et al., 2006; Muller and Chittka, 2012; Junca et al., 2014; Smith and Raine, 2014; Tait et al., 2019) can be described in terms of a speed-accuracy trade-off (Chittka et al., 2003). The functional consequences of this trade-off are, however, ambiguous—while one study suggested that fast and inaccurate foragers, which are more indiscriminate in their choice between rewarding and non-rewarding flowers, can collect resources at a higher rate (Burns, 2005), another showed that such foragers end up with a lower lifetime collection (Evans et al., 2017). Although the results of these two studies are not easily comparable given their different experimental designs, they suggest that slow and fast cognitive strategies might have different costs and benefits associated with them. Colonies that maintain a cognitively diverse workforce therefore might be able to show an overall higher efficiency in resource acquisition (Burns and Dyer, 2008).

The positive influence of cognitive diversity on collective foraging is most likely mediated by the resource landscape, which has a strong influence on foraging dynamics (Waddington et al., 1994; Steffan-Dewenter and Kuhn, 2003; Couvillon et al., 2015). Since slow and fast cognitive phenotypes differ with respect to how they gather and use information, a more challenging or scarce resource environment can enhance the value of producing new information, while a more rich or clumped resource environment can reduce its value and provide an advantage for scrounging. In a social foraging context, the collective performance of the group can therefore be viewed in the framework of a Genotype × Environment interaction, where the genotype specifies the relative frequency of each cognitive phenotype in the group and the environment refers to the spatial and temporal distribution of resources. Despite the long history of theoretical work on this topic (Caraco and Giraldea, 1991; Vickery et al., 1991; Luttbeg and Sih, 2010), actual empirical tests of how the performance of different cognitive strategies is influenced by the resource environment are extremely rare, largely because of their challenging nature (King et al., 2009).

COMPARATIVE COGNITION: EVOLUTION OF SLOW-FAST COGNITIVE PHENOTYPES

Our understanding of the functional relevance of cognitive variation can strongly benefit from studies at the interspecific level (Chittka et al., 2012; Rosati, 2017; Wenseleers and van Zweden, 2017). Although the initial studies that placed behavioral and life history traits along a slow-fast axis made use of interspecific comparisons (Promislow and Harvey, 1990; Wiersma et al., 2007), the same approach has not been used as much to understand similar variation in cognitive traits across species. Since interspecific differences in cognition represent adaptations to differences in ecological factors (Healy et al., 2009; Sheehan and Tibbetts, 2011; Cauchoix and Chaine, 2016), understanding such differences in terms of a slow-fast axis would be an important step toward identifying its evolutionary significance. In the context of a social group, we know little regarding whether the link between individual and collective cognition is modulated by ecological factors.

However, interspecific comparisons of cognitive traits can be confounded by the fact that any observed differences across species could be attributed to assays or experimental conditions that are not equally relevant for each species. One therefore needs to exercise good judgment in terms of the experimental design and the species that are chosen for such studies. It has also been pointed out that seemingly similar cognitive capacities might be mediated by entirely different neural mechanisms and therefore behavioral studies must be complemented with research at a proximate level (Chittka et al., 2012). Neuroecology, the comparative study of mechanisms that underlie cognitive capacity, has provided robust support for the adaptive nature of cognitive differences in social insects in terms of brain evolution (Kamhi et al., 2016; Godfrey and Gronenberg, 2019). Based on these findings that support the classic idea that social evolution is one of the strongest drivers of brain evolution (Dunbar, 1998; Dunbar and Shultz, 2007), social insects are particularly suited to understanding how cognitive trait evolution is related to sociality.

Although honeybees have served as a classic model of cognition research (Menzel, 2012; Giurfa, 2015), our extensive knowledge regarding their cognitive capacity is largely derived from studies with *Apis mellifera*, with little known about the cognitive traits of other honeybee species. Comparative studies of cognition across these different species offer an excellent, yet untapped, experimental opportunity that can give insights into the role of ecological factors on cognitive variation at both the individual and the collective levels. The two cavity nesting species, *A. mellifera* and *A. cerana*, have been described as "fast" due to their shorter lifespan, higher metabolic rate and faster behavioral "tempo" compared to the two open nesting species, *A dorsata* and *A. florea*, described as "slow" (Seeley, 1985; Dyer and Seeley, 1991). In fact, Seeley (1985) speculated about clusters of functionally related traits that could identify causal relationships between ecology and social organization and how such a goal could be realized with a comparative trait-oriented approach across the four species. The slow-fast phenotypic axis offers exactly that opportunity in terms of a unifying framework that can be used to measure a large set of traits at different phenotypic levels and systematically test if the slow-fast differences among these species also extend at the level of cognition and if the covariance among traits is shaped by the ecological differences among these species.

A comparative approach inspired by this framework was recently used to test if the observed slow-fast cognitive differences among *A. mellifera* individuals (Tait and Naug, 2020) are consistent across the other honeybee species and if there are slow-fast cognitive differences among these species that match differences in their behavior, life history and ecology (Tait et al., 2021). The results suggest some consistency in the traits that define the slow-fast cognitive axis within each of the four species—specifically, individuals which are fast learners also show higher preference for novelty compared to those who are slow. However, interspecific differences in cognitive traits did not correlate to slow-fast differences in life history and nesting ecology as it was *A. florea* (slow "tempo") and *A. cerana* (fast "tempo") which were found to cluster together as a distinct group, characterized by their lower associative learning and higher risk preference than *A. dorsata* (slow "tempo") and *A. mellifera* (fast "tempo") which formed a separate cluster. Instead, it was found that these interspecific cognitive differences correlate to differences in absolute brain size—*A. dorsata* and *A. mellifera*, the two species with higher associative learning also have significantly larger brains than *A. cerana* and *A. florea* (Gowda and Gronenberg, 2019; Tait et al., 2021). Although the analysis of this observed relationship is somewhat rudimentary, these results highlight the importance of integrating measurements from multiple phenotypic levels to understand the basis of cognitive differences across species. The two species with larger brains, *A. dorsata* and *A. mellifera*, also have both a larger colony size and a more complex foraging niche in terms of its spatiotemporal complexity, compared to *A. florea* and *A. cerana* (Seeley, 1985; Dyer and Seeley, 1991). It is therefore worth asking if the evolution of slow-fast cognitive traits is related to social and environmental complexity, which are widely recognized as important drivers of cognitive evolution (Roth and Pravosudov, 2009; Roth et al., 2010), which includes evidence from social insects (Farris and Schulmeister, 2011; Farris, 2016; Kamhi et al., 2016).

ENERGETICS OF COGNITION: MECHANISTIC BASIS OF SLOW-FAST COGNITIVE PHENOTYPES

If brain size is an important determinant of cognitive capacity (Chittka and Niven, 2009), it becomes important to understand the neural mechanisms that shape slow-fast differences in cognitive traits. There are substantial energetic costs associated with neural processing and differences in cognitive capacity are predicted to be fundamentally derived from variation in brain metabolic activity (Laughlin et al., 1998). This mechanistic connection between cognitive capacity and energy use allows us to test if slow-fast differences in cognitive traits are shaped by differences in metabolic rate at both the intra- and interspecific levels. Combined with the knowledge that behavioral traits such as aggression are also correlated to metabolic activity in the brain (Rittschof et al., 2018), this will help connect the slow-fast cognitive axis to the common energetic link that characterizes the broader pace-of-life axis (Careau et al., 2008; Mugel and Naug, 2020).

In honeybees, energetic availability can drive differences in associative learning (Jaumann et al., 2013), risk sensitivity (Mayack and Naug, 2011), exploration-exploitation tendency (Katz and Naug, 2015, 2016) and impulsivity (Mayack and Naug, 2015), each of which is part of the slow-fast cognitive axis. A growing body of work shows that differences in associative learning and memory are robustly related to metabolic activity in the brain, as measured with cytochrome oxidase (COX), a metabolic marker of neuronal activity (Déglise et al., 2003). The well-known link between cognitive differences and levels of various neurotransmitters (Mercer and Menzel, 1982; Giurfa, 2006; Cook et al., 2019) could also be reflective of such differences in brain metabolic activity since energy use is regulated through the same signaling pathways (Roeder, 2020). By combining measurements of variation in both whole-body metabolic rate and brain ATP capacity with performance on cognitive assays, one can test whether the slow-fast differences in cognitive phenotypes are fundamentally related to a difference in metabolic capacity.

A link between cognitive capacity and energy use suggests that the collective cognitive capacity of a social group would be fundamentally influenced by the variation in metabolic rate within the colony. However, such a relationship can be complicated by the fact that collective cognition is proposed as a mechanism for relaxing the energetic constraints on cognition at the individual level (Lihoreau et al., 2012; Feinerman and Traniello, 2016; Feinerman and Korman, 2017; Coto and Traniello, 2021). In social insects, increasing social complexity is correlated to a decrease in brain size at the individual level and it is argued that distributed cognition can allow for investment in functionally specialized brain regions, ultimately lowering brain metabolic costs (O'Donnell et al., 2015). In ants, socially complex species have larger brains but a lower energetic activity in the higher order processing centers such as mushroom bodies (Kamhi et al., 2016). These results suggest that individual energetic constraints related to learning and other slow-fast cognitive differences may not be reflected as easily in social insects and emphasizes the importance of identifying the mechanisms that link individual cognitive capacity to collective cognition.

COGNITIVE PLASTICITY: DEVELOPMENTAL ANALYSIS OF SLOW-FAST COGNITIVE PHENOTYPES

What adds to the difficulty of understanding and defining the cognitive capacity of an individual is its plasticity, subject to influences from both the current and the developmental environment (Thornton and Lukas, 2012; Buchanan et al., 2013; van den Bos et al., 2013; Davidson et al., 2018; Cauchoix et al., 2020). These include influences of the nutritional, physical and social components of the environment, which acting through various epigenetic modifications and signaling pathways, can shape brain development, function and neural plasticity (Murphy et al., 2014). Environmental variability plays a major role in the covariance among different traits (Sgrò and Hoffmann, 2004; Wright et al., 2019) and it is proposed that the predicted slow-fast trait correlations are more likely to be observed in unfavorable environments (Hämäläinen et al., 2021). Such developmental effects on the adult phenotype can be either plastic (permanent environment effects) or flexible in the short term (reversible plasticity). Developmental effects may be adaptive if they result in a phenotype that is better fitted to the environment the individual is likely to experience as an adult—the so called Predictive Adaptive Response (PAR) hypothesis, according to which a match between the developmental and the adult environments leads to positive effects and a mismatch leads to adverse effects on various phenotypic traits (Gluckman and Hanson, 2004). Despite the strong support for such developmental effects on various phenotypic traits, including some work on honeybees (Wang et al., 2016), studies focusing on the patterns of slow-fast cognitive traits in an environmental context are rare.

Developmental effects are likely to be particularly relevant in social insects because the age-based division of labor is a developmental process that is both plastic and is accompanied by several important changes in cognitive traits (Ben-Shahar et al., 2000; Cabirol et al., 2018). In honeybees, several studies show the influence of birth weight and early social experience on sucrose responsiveness and associative learning (Pankiw et al., 2004; Scheiner, 2012; Arenas et al., 2013; Mortensen and Ellis, 2018; Tsvetkov et al., 2019) but we lack the knowledge about similar effects on other cognitive traits. The use of social information can be shaped by resource unpredictability during development, pre-natal stress leading to copying behavior in adulthood and post-natal stress leading to the opposite effect (Boogert et al., 2013). Since social information use is one of the key parameters that defines the scout-recruit behavioral axis or the slow-fast cognitive axis, these results suggest promising research avenues to understand the inconsistencies that are sometimes seen in the expression of these cognitive phenotypes. Developmental effects on cognitive traits are especially relevant in the current context of the influence of anthropogenic changes and other stressors on behavior (Decourtye et al., 2005; Gómez-Moracho et al., 2017).

CHALLENGES AND SOLUTIONS: A ROADMAP FOR FUTURE WORK

Methodological Approaches

One of the most challenging aspects of establishing a detailed and robust cognitive axis is the measurement of multiple cognitive traits in multiple contexts, made even more difficult in social insects due to the relatively short worker lifespan. Perhaps one of the most appealing aspects of using honeybees as a model system for the study of cognitive variation is the ability of the well-established laboratory-based Proboscis Extension Reflex (PER) assay to measure several cognitive traits in a large number of individuals with a high throughput. The PER assay consists of presenting a bee with an odor, a conditioned stimulus (CS), followed by a sucrose reward, an unconditioned stimulus (US), in a series of trials and measures learning ability as the number of conditioned responses (CR), instances when the bee extends its proboscis to the CS prior to the US delivery (Bitterman et al., 1983). While this assay is conventionally used for measuring various types of learning, including discrimination learning (Smith et al., 1991) and reversal learning (Chandra et al., 2000), it can also be adapted to measure a variety of more complex cognitive traits such as risk-sensitivity (Shafir et al., 1999; Mayack and Naug, 2011), sampling and novelty preference (Katz and Naug, 2015; Tait and Naug, 2020). These assays have also been adapted for use in the other honeybee species (Ali et al., 2021; Tait et al., 2021), an important consideration if we are to extend the comparative study of cognitive phenotypes in honeybees. The appetitive PER assay, and the closely similar, aversive Sting Extension Reflex (SER) assay (Vergoz et al., 2007), also allow us to control for differences in motivational state that might otherwise confound the measurement of cognitive traits in field-based assays.

Experimental evolution and artificial selection approaches can be powerful tools in uncovering how a phenotypic trait might be shaped by specific selection pressures. Studies in cognitive variation have, however, lagged in this regard probably due to the large number of traits that are required to define the cognitive axis, their plasticity and the likely small heritability component in these traits due to the complex genetic architecture of cognition. The honeybee, being the only social insect that has been successfully bred for specific traits, allows extraordinary opportunities to select and breed for specific phenotypic traits using instrumental insemination techniques. Using the heritable variation in associative learning (Brandes, 1988, 1991; Laloi and Pham-Delegue, 2010), genetic lines with differences in their associative learning or reversal learning ability have been bred (Brandes and Menzel, 1990; Bhagavan et al., 1994; Chandra et al., 2000; Ferguson et al., 2001). Such cognitive lines can allow us to rigorously test the nature of the covariance structure among different cognitive traits and partition it into genetic and environmental effects. These lines can enable us to test the functional influence of various cognitive traits at the individual level as well as allow the possibility of creating experimental mixes of different cognitive phenotypes to test how cognitive differences scale up from the individual to the collective level to influence group-level performance and life history (Cook et al., 2020).

Statistical Approaches

Cognitive phenotypes, defined as consistent individual differences in several cognitive traits, also pose a challenge in terms of statistical analysis. Since these phenotypes are described by multiple traits that covary with each other, the complexity of the multivariate cognitive trait space needs to be captured by data reduction techniques such as principal component analysis (PCA), which can be used to identify specific cognitive traits that explain the largest differences among different cognitive phenotypes (Keagy et al., 2011; Mazza et al., 2018; Tait and Naug, 2020). PCAs, used to study interindividual variation *within* a species, however, are not appropriate when comparing the variation *between* species because individuals of a species are more similar to each other than those from other species. A related technique, canonical variate analysis (CVA), which maximizes the separation of *a priori* defined groups, rather than the individuals within each of them, is more appropriate for such interspecific comparisons (Campbell and Atchley, 1981; Carter and Feeney, 2012; Tait et al., 2021). Such statistical non-independence arising from phylogenetic inertia (Harvey and Purvis, 1991; Sherry, 2006) could also be addressed by using a phylogenetically corrected analysis such as the phylogenetic generalized least squares (PGLS) method (Székely et al., 2013). With the measurement of multiple cognitive traits, it also becomes important to understand the causal relationships among these different traits and the use of Structural Equation Models (SEM) or path analysis can allow us to extract such relationships. Studies on cognitive phenotypes, which mostly rely on simple covariance analyses, need to see a wider adoption of these more sophisticated statistical approaches.

In terms of collective performance, groups are generally compared using statistical models that are focused on the parameters of mean or variance. Such analyses are, however, unable to offer any insights into the possible mechanisms that mediate the effects of phenotypic diversity. In this context, a rarely used technique based on the Price equation, which identifies the different mechanisms that underlie the effects of diversity more specifically, can be used to analyze the influence of diversity on performance (Loreau and Hector, 2001; Takahashi et al., 2018). In this approach, the effect of inter-individual variation or heterogeneity on group performance is quantified as a diversity effect, which is then further partitioned into a complementarity effect, the influence of interactions between different phenotypes, and a selection effect, the disproportionate influence of a phenotype. A recent study used this technique to explore the influence of metabolic diversity in honeybees to find that the effects of diversity on collective performance can be complex (Mugel and Naug, in press) and it remains an exciting prospect to use a similar approach in studies of cognitive diversity.

Modeling Approaches

The speed-accuracy trade-off, which is considered as the underlying basis for the slow-fast cognitive axis, is a classic paradigm that addresses how animals manage these two constraints at the same time in a manner that maximizes the benefit to cost ratio of a decision. Numerous models describing such decision-making processes show a parallel between individual and collective decision-making where populations of neurons or individuals accumulate evidence for alternative choices and a decision is made for a specific alternative once the population reaches a threshold for that alternative (Bogacz, 2007; Marshall et al., 2009; Pelé and Sueur, 2013). These decision-making models are therefore more fundamentally tied to models of optimal sampling and learning, which predict that an animal should sample more and therefore learn more slowly, but more accurately, if the cost of making a wrong decision is large or if the cost of waiting to make a decision is low, both of which in turn are tied to the variability in the environment (Stephens, 1987, 1989, 1991). Sampling and the statistical property of the central limit theorem (CLT), which posits that the estimate of the true mean (accuracy) improves with sample (group) size, is also what explains the improved performance of a collective unit as against an individual. However, it is important to note that this outcome of CLT is based on the assumption of a random sample, which in this context would refer to a random assortment of cognitive phenotypes. Using specific distributions to model cognitive heterogeneity within a group, it would be instructive to generate testable predictions about how diversity of slow-fast cognitive phenotypes would influence the sampling process and how that in turn would affect the collective cognitive performance of the group.

Since the basis of collective cognition lies in a group of heterogeneous individuals interacting together, it has frequency-dependent outcomes that can be modeled using a game-theoretic approach, which allows us to predict the performance of each morph based on its relative frequency and how that in turn shapes the performance of the entire group. While the inclination to learn can be strongly influenced by the frequency of learners and non-learners in a group, only a handful of studies has examined the evolution of learning or any other cognitive parameter and its consequence on collective performance in a game-theoretic context (Giraldeau, 1984; Dubois et al., 2010; Katsnelson et al., 2012; Aplin and Morand-Ferron, 2017). Modeling approaches like these can be productively combined with statistical approaches that can analyze the details of the diversity effects in empirical data as described above. The scarcity of studies in this framework probably stems from the fact that we largely lack the ability to create experimental groups with specific compositions of different cognitive phenotypes, an endeavor which can be accomplished by the ability to generate selection lines as discussed earlier.

Finally, we feel that a major, but mostly overlooked, problem in the field of animal personality and animal cognition in general, is a lack of coherent and consistent definitions of different traits. Overlapping definitions sometimes lead to the same traits being measured in different ways, which means that they are not statistically independent of each other and can lead to inconsistent patterns about how these traits covary. For example, many studies measure exploration, activity and neophilia as three independent "eco-cognitive behavioral traits," but unless defined and assayed carefully, the magnitude of these three traits could interdependently follow from each other. A lower preference for novelty could also be expressed as a lower level of sampling, which in turn may also lead to poorer learning, a covariance structure that is not only non-independent but also one that does not exactly match both model predictions (Sih and Del Giudice, 2012) and empirical data (Tait and Naug, 2020) about slow-fast cognitive phenotypes. Similarly, "reaction to a novel object" is frequently used as a definition of exploration, but it is also used as a measure for boldness even though the latter might be more specifically to do with an individual's reaction to a risky situation. Risk itself is defined in terms of tendency of an individual to expose itself to a predator (Ferrari, 2014) or a preference for variability (Tait and Naug, 2020; Tait et al., 2021). The biological mechanisms driving these diverse behaviors could be very different and might lead to very different predictions about how they might co-vary with each other. This problematic issue is partly a consequence of the fact that cognitive processes are not directly measurable and can only be inferred by assessing a change in behavior (Barron et al., 2015; Griffin et al., 2015; Mazza et al., 2018). In this setting, process-based models can be informative about how these traits might be linked to each other, allowing us to see if these numerous traits are outcomes of a single fundamental cognitive process, such as sampling, or if they can vary independently of each other.

These obfuscations about how different cognitive traits are defined and measured point to a larger problem of how a set of cognitive traits is predicted to covary with each other to define different cognitive phenotypes. Models of the speed-accuracy trade-off, which is used as the major framework to conceptualize decision-making and the existence of slow but accurate and fast but inaccurate cognitive phenotypes (Sih and Del Giudice, 2012), have not considered broader and influential ideas regarding trade-offs. According to life history theory, trade-offs are a combined outcome of differences in both resource acquisition and allocation such that negative correlations between traits are produced only when individuals vary mainly in their allocation while positive correlations between the same traits can be seen when individuals vary mainly in their acquisition (Van Noordwijk and De Jong, 1986; Reznick et al., 2000). Since

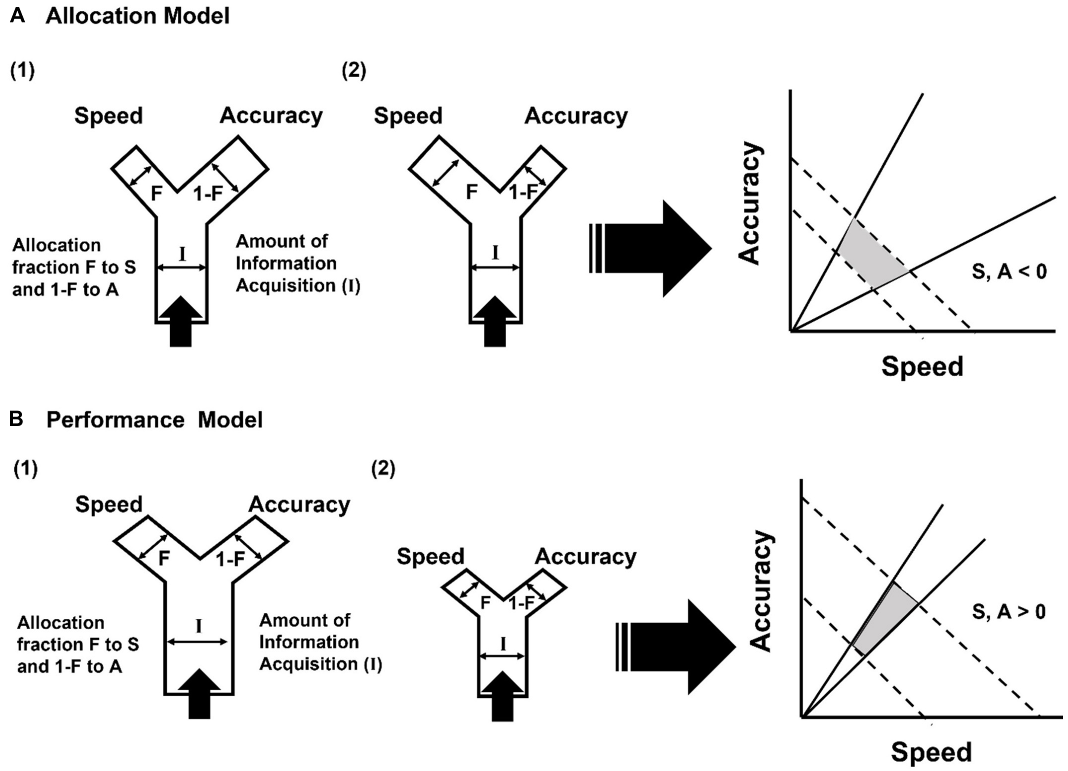

FIGURE 3 | Possible alternative Y models of information (I) acquisition and its allocation to speed (S) and accuracy (A) in terms of either **(A)** an allocation model in which there is low inter-individual variation in information acquisition but high variation in its allocation, resulting in a scenario where (1) individuals invest more in accuracy at the expense of speed, or (2) *vice versa*, resulting in a negative relationship between speed and accuracy, or **(B)** a performance model in which there is high inter-individual variation in information acquisition but low variation in its allocation, resulting in a scenario where (1) individuals have a greater total amount of information that can be allocated to speed and accuracy, or (2) individuals have a lower total amount of information that can be allocated to speed and accuracy, resulting in a positive relationship between speed and accuracy.

information can be considered as a resource, the same framework also applies in the context of decision making (**Figure 3**). Therefore, if individuals vary mainly in their ability to *acquire* information, as it is likely to be if it is correlated to traits such as metabolic rate and energetic capacity as might be expected from the pace-of-life hypothesis, it can produce a positive correlation between speed and accuracy, which might explain the seemingly contradictory absence of a trade-off between speed and accuracy during learning in some studies (Raine and Chittka, 2012; Mamuneas et al., 2015; Chang et al., 2017). Only when individuals similar in their information acquisition ability vary in how they allocate this information, should one see the more commonly expected trade-off between speed and accuracy. Since differences in acquisition are more likely to be manifested in some environments than others (Reznick et al., 2000), it means that the expression of the speed-accuracy tradeoff could be restricted to specific types of information environments. This also implies that the nature of covariation among different cognitive traits could be driven by Gene x Environment interactions, leading to possible polymorphisms in terms of different suites of covarying cognitive traits.

CONCLUSION

Collective cognition has long been a topic of major interest to biologists and information scientists and a large body of both theoretical and empirical work has firmly established that groups can generally acquire and process information more efficiently and accurately than it is possible for single individuals. These findings about collective cognition closely parallel what is seen for collective traits at other phenotypic levels such as behavior and physiology so that it is now an established fact that collective action generally results in more robust outputs that are more stable to perturbations. Given this background, we feel that it is time now to move beyond this and take the next major step toward integrating these findings to a broader theoretical framework that can connect cognition to other levels of phenotypic variation at multiple levels of biological organization. The pace-of-life framework, which aims to place suites of phenotypic traits including cognition on a common slow-fast axis, seems particularly suited to this enterprise, allowing us to understand both the mechanistic and functional integration across these traits, which is important if we are to understand the role of cognition in collective behavior and social evolution. Applying the framework at the group level allows us to understand how the constraints posed upon the individual by the covariance among different cognitive traits might be relaxed by collective action. Social insects, especially honeybees, due to their wide-ranging experimental amenability, provide ideal model systems to apply this framework in testing how the slow-fast cognitive composition of a group shapes the emergent collective cognitive phenotype to influence colony behavior and life history.

AUTHOR CONTRIBUTIONS

DN and CT conceived and wrote the manuscript together. Both authors contributed to the article and approved the submitted version.

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Caste, Sex, and Parasitism Influence Brain Plasticity in a Social Wasp

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Brain plasticity is widespread in nature, as it enables adaptive responses to sensory demands associated with novel stimuli, environmental changes and social conditions. Social Hymenoptera are particularly well-suited to study neuroplasticity, because the division of labor amongst females and the different life histories of males and females are associated with specific sensory needs. Here, we take advantage of the social wasp *Polistes dominula* to explore if brain plasticity is influenced by caste and sex, and the exploitation by the strepsipteran parasite *Xenos vesparum.* Within sexes, male wasps had proportionally larger optic lobes, while females had larger antennal lobes, which is consistent with the sensory needs of sex-specific life histories. Within castes, reproductive females had larger mushroom body calyces, as predicted by their sensory needs for extensive within-colony interactions and winter aggregations, than workers who frequently forage for nest material and prey. Parasites had different effects on female and male hosts. Contrary to our predictions, female workers were castrated and behaviorally manipulated by female or male parasites, but only showed moderate differences in brain tissue allocation compared to non-parasitized workers. Parasitized males maintained their reproductive apparatus and sexual behavior. However, they had smaller brains and larger sensory brain regions than non-parasitized males. Our findings confirm that caste and sex mediate brain plasticity in *P. dominula*, and that parasitic manipulation drives differential allocation of brain regions depending on host sex.

Keywords: brain plasticity, parasite, parasitic manipulation of host, *Polistes dominula*, sensory brain regions, social wasp, strepsiptera, *Xenos vesparum*

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INTRODUCTION

Brain plasticity enables adaptive responses to different sensory demands such as novel stimuli, changing environments and social conditions (Taborsky and Oliveira, 2012; O'Donnell et al., 2013; Anderson and Finlay, 2014; Kamhi et al., 2017; Jernigan et al., 2021). For instance, neuroplasticity has evolved across many lineages as a result of variable selective pressures acting on the cognitive demands of sensory and perceptual systems (Barton et al., 1995; Barton and Harvey, 2000; Catania, 2005). Since brain tissue is energetically expensive, plasticity in specific brain structures may be linked to the sensory and processing needs of adaptive behaviors (Isler and Van Schaik, 2006;

Niven and Laughlin, 2008; Riveros and Gronenberg, 2010; Pyza, 2013; Montgomery et al., 2016; Dunbar and Shultz, 2017; Rozanski et al., 2021). Investment in neural tissue may be mediated by experience (Jones et al., 2009; Riveros and Gronenberg, 2010; Cabirol et al., 2018), diet (Murphy et al., 2014; DeCasien et al., 2017), environmental stimuli (Burns et al., 2009; Axelrod et al., 2018), tradeoffs with reproduction (Pitnick et al., 2006), and/or endocrine factors (Ball et al., 2002; Lendvai et al., 2013). Previous studies also provide compelling evidence for how neuroplasticity adaptively supports the division of labor in complex insect societies and matches sensory specialization (Groh and Meinertzhagen, 2010; Giraldo et al., 2013; Kamhi et al., 2017; Gordon et al., 2019; Arganda et al., 2020; Baudier et al., 2021).

Given that specialized behaviors are associated with a range of caste-specific sensory needs, corresponding investment in neural tissue is expected (Gronenberg et al., 1996; Ehmer et al., 2001; O′Donnell et al., 2007; Seid et al., 2011; Rehan et al., 2015; Arganda et al., 2020; Penick et al., 2021). Therefore, social insects provide the opportunity to compare differential investment in sensory brain regions between castes and sexes with different life histories (Gronenberg and Riveros, 2009; Beani et al., 2014), while controlling for genetic background. In insect brains, visual input travels from the eyes and is received and processed by the optic lobes, while olfactory input is received by the antennal lobes (Strausfeld, 1989; Anton and Homberg, 1999; Gronenberg and Hölldobler, 1999). From these lower-order sensory neuropils, projection neurons convey the computed information to the mushroom bodies (Akalal et al., 2006). In these higher-order brain centers, the chemical and visual information is further processed and integrated with internal information by intrinsic neurons and finally projected to premotor areas. The substructures of the mushroom body calyces, act as learning and memory centers that integrate sensory information and foraging experience. Specifically, olfactory information is processed in the lip, the visual information in the collar, and both sensory stimuli in the basal ring (Ehmer and Hoy, 2000; Akalal et al., 2006; Fahrbach, 2006). Finally, the central complex is implicated in spatial navigation (Pfeiffer and Homberg, 2014; Honkanen et al., 2019; Le Moël et al., 2019).

Here, we take advantage of the primitively eusocial paper wasp *Polistes dominula* to test how brain plasticity is associated with behavioral flexibility (Pardi, 1996; O'Donnell et al., 2014, 2018; O'Donnell and Bulova, 2017; Rozanski et al., 2021). In this temperate wasp species, the recognition of nestmates, caste, and sex relies on both chemical and visual cues (Dani et al., 2001; Cappa et al., 2016, 2020; Beani et al., 2019; Cini et al., 2019). Females are morphologically similar and organized in a flexible caste system, according to a dominance hierarchy (Pardi, 1948). The reproductive castes emerge in mid-summer and consist of males and gynes that will become foundresses the following spring. Gynes remain on the natal nest without performing any colony tasks and then mate, form winter aggregations, and enter diapause (Reeve, 1991). The following spring, foundresses initiate construction of nests and compete for the dominant position establish a dominance hierarchy. The dominant foundress monopolizes egg-laying, and the first offspring will become workers (Strassmann et al., 2004). Therefore, subordinate foundresses and workers are involved in nest building and defense, the rearing of larvae, and foraging (West-Eberhard, 1969). In the mid-summer, adult males and new gynes emerge. Males abandon the nest early after emergence and display lek-behavior at landmarks where they mate with gynes and die at the end of fall (Beani, 1996; Beani et al., 2014). Gynes store sperm for reproduction during the following spring (Cappa et al., 2013).

In addition, *P. dominula* is also parasitized by the strepsipteran insect *Xenos vesparum*, which provides a great opportunity to explore the effect of this parasite in allocation of brain tissue (Hughes and Libersat, 2018; Libersat et al., 2018). *X. vesparum* larvae enter worker wasp larvae in the early summer and develop inside their hosts (Manfredini et al., 2010). When the hosts emerge as adults, the parasites undergo pupation and behaviorally manipulate their hosts (Hughes et al., 2004b). After metamorphosis, adult female parasites remain as obligate endoparasites inside the host, while adult males emerge from the host, and mate with females. Parasite pupae decrease the size of corpora allata in female hosts and castrate them by irreversibly inhibiting ovary development (Strambi and Strambi, 1973; Strambi et al., 1982; Hughes et al., 2004b; Beani, 2006). Parasitized workers abandon the colony and aggregate on selected plants where parasite mating occurs (Hughes et al., 2004b; Beani et al., 2018). In contrast, male wasps are less-frequently parasitized, and instead maintain their reproductive apparatus and sexual behavior (Beani et al., 2011; Cappa et al., 2014). After mating, female parasites extend the lifespan of their worker host to overwinter like a gyne (Beani et al., 2021). Instead, female and male hosts parasitized by males die at the end of the summer (Beani et al., 2021).

While brain plasticity within and across social insects has been extensively studied (Godfrey and Gronenberg, 2019), no studies have explored plasticity within a species that has morphologically similar individuals, various colony tasks, and a parasite that potentially alters brain morphology. We predicted that the relative volume of selected brain regions reflects specific sensory needs for each caste and sex (reproductive females, female workers, and males) (Rozanski et al., 2021). We also predicted higher volume of visual regions in males to detect and identify potential mates or rival males in a lek, compared to females. On the contrary, we expected more olfactory processing by females compared to males due to social interactions in the colony. We also tested for the effect of parasitic manipulation in brain allometry. We predicted a stronger parasite effect in the brain of workers, because they are castrated and show strong behavioral manipulation, compared to parasitized males who reproduce and show no changes in behavior. Finally, little is known about the specific neuroendocrine effects of female and male parasites toward female and male hosts. Based on the strong behavioral alterations induced by the parasite on worker wasps (Strambi and Strambi, 1973; Beani et al., 2017), we expected a reduction of corpora allata regardless of parasite sex. Conversely, given the mild parasite impact on male hosts (Cappa et al., 2014; Beani et al., 2017), we predicted a small effect of *X. vesparum* on male corpora allata size.

MATERIALS AND METHODS

Field Collection

We collected reproductive females ($N = 10$ foundresses and $N = 9$ gynes), non-parasitized workers ($N = 10$), workers parasitized by one *X. vesparum* female ($N = 11$) or by one *X. vesparum* male ($N = 11$), non-parasitized males ($N = 10$), and males parasitized by one or two *X. vesparum* males ($N = 9$). All samples were collected during the first days of July of 2016 and 2018, in the plain of Sesto Fiorentino (Tuscany, Italy). Males parasitized by *X. vesparum* females and parasitized gynes are lacking in our data set, due to the protandrous emergence of *X. vesparum* (Hughes et al., 2004a), and to the scarcity of male and gyne reproductive larvae during the infection period in the early summer. Wasps from each caste emerge synchronously and at specific times throughout the summer, which controls for age (Molina and O'Donnell, 2008) and seasonality effects that can influence brain development. Non-parasitized and parasitized hosts are easily distinguished by inspecting for parasite extrusions between the abdominal tergites, and parasites can be identified as female or male because of the shape of their pupal sac (**Figure 1B**). Finally, to verify which individuals were parasitized, their abdomens were preserved and dissected in 70% ethanol. We confirmed the absence of pupal parasites in female workers and males without any visible signs of parasitism. In parasitized workers and males, we also confirmed sex of the parasites and gonad development predicted for each category (**Figure 1C**). Finally, we preserved each head capsule individually in a glyoxal fixative for subsequent histological sectioning (Prefer, Anatech Ltd., Battle Creek, United States).

Histology and Measurement of Brain Regions

We first dehydrated each head capsule with a series of increasing ethanol and acetone concentrations. We then used the established concentrations for the Embed 812 resin kit (Electron Microscopy Sciences, Hatfield, United States) to embed the head capsule while maintaining their brain dimensions, following the histology protocol for *Polistes* wasps (O'Donnell et al., 2015; Rozanski et al., 2021). The samples were moved repeatedly between an open-air rocking shaker (Thermo Fisher Scientific, Waltham, United States) and a vacuum to improve infiltration of the solvent.

Next, we placed each embedded head capsule in an individual plastic mold filled with the same concentration of resin in an oven at 60°C. After 72 h, the resin was polymerized. We sectioned each brain in consecutive coronal sections with a thickness of 17 μm and stained the tissue with toluidine blue, to visualize clearly defined boundaries for each brain region. We photographed the consecutive brain sections for each specimen using a Canon EOS 5D Mark III mounted on a Leica DM IL LED microscope at 4 x magnification, including a scale of 1,000 μm.

Using the AxioVision SE64 (Zeiss, NY, United States), we outlined the area for each individual brain region (**Figure 1A**). We traced the antennal lobes and the three substructures of the optic lobes: medulla, lobula and lamina. We also traced the two calyx substructures process olfactory and visual stimuli: lip and collar, respectively, and the central complex. The remaining structures in the protocerebrum were grouped as the central brain (**Figure 1A**), following the established method for this species (Rozanski et al., 2021) and ants (Sheehan et al., 2019). Outlining of brain regions was done blind to the category for each sample. We quantified each brain region for every other section per brain, as this method shows high accuracy (i.e., < 3.5% error for 34 μm thick sections) (Ehmer and Hoy, 2000). We then determined the volume for each region by multiplying the area by the distance between sections (34 μm). We generated the 3-D brain reconstruction by using the software RECONSTRUCT (Fiala, 2005). To control for the effect of head size, we measured head width. Finally, we determined the cross-sectional area of the corpora allata by measuring the diameter of one of the two glands, following the method previously used for this species (Strambi and Strambi, 1973).

Statistical Analyses

We explored if differential volume in specific brain regions among phenotypes was the result of changes in allometric scaling (Ott and Rogers, 2010; Eberhard and Wcislo, 2011; Seid et al., 2011; O'Donnell et al., 2013; Stöckl et al., 2016; Sheehan et al., 2019). In *P. dominula*, the optic lobe represents on average 42% of the brain and may have an effect on relative neuropil scaling (Rozanski et al., 2021). Therefore, we compared investment in each sensory brain region to the central brain, instead of by the whole brain (Ott and Rogers, 2010; Stöckl et al., 2016; Sheehan et al., 2019).

We used the allometric equation $y = a^*x^\beta$ for the scaling relationship between brain regions x and y. We then logarithmically transformed the estimates β (slope) and α (intercept of a regression) by using the linear equation $\log(y) = \beta\log(x) + \log(a)$, where $\log(a) = \alpha$ (Dubois, 1897; Huxley and Teissier, 1936). Standardized Major (SMA) regression analyses were calculated by using the SMATR v.3 package for R (WartonI, Wright et al., 2006, Warton et al., 2012).

First, we tested for a common slope among non-parasitized phenotypes as a baseline comparison, consisting of males, reproductive females and workers ($H^0 = \beta_{males} = \beta_{reproductives} = \beta_{workers}$). We implemented log-likelihood tests followed by *post hoc* pairwise comparisons provided in the SMATR package. Since allometric scaling did not differ significantly between foundresses and gynes, we pooled them under a new category called "reproductives." Second, we tested for a common slope among non-parasitized workers, with one female parasite and with one male parasite, and between non-parasitized and parasitized males. The volume of brain regions did not differ between male wasp parasitized by one or two male *X. vesparum*, so we also pooled them. We compared allometric changes in the whole brain with head width, central brain with whole brain, and pooled sensory regions with changes in the central brain. Finally, we explored the allometric relationship between each sensory brain region and central brain, following our established method for this wasp species (Rozanski et al., 2021).

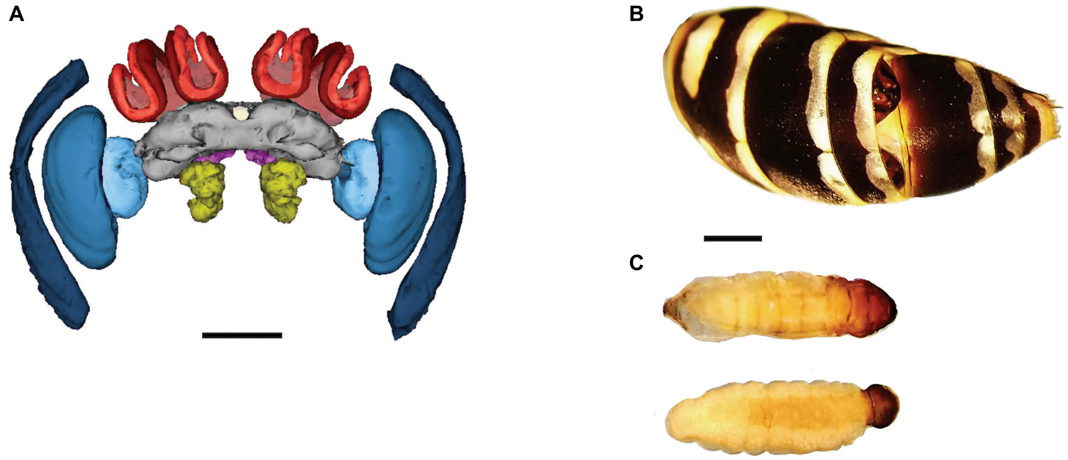

FIGURE 1 | Study system: a female brain, the abdomen of a parasitized female, and *Xenos vesparum* parasites. **(A)** Frontal view of a 3-D reconstructed brain of a female *Polistes dominula.* The sensory brain regions are color coded: substructures of the optic lobes (blue), antennal lobes (yellow), lip (bright red), collar (dark red) and the central complex (white). All sensory regions are normalized by the central brain (gray). For reference, the subesophageal zone is shown in bright pink and the mushroom body peduncles in light red. Scale bar = 500 μm. **(B)** A host abdomen shows a male *X. vesparum* pupa (top) and the cephalothorax of a neotenic female *X. vesparum* pupa (bottom) extruding from the tergites. **(C)** Same larvae of *X. vesparum* after being dissected from the host's abdomen: male parasite on top and female on the bottom. **(B,C)** Are scaled (scale bar = 2 mm).

For categories that shared a common slope, we used log-likelihood tests to calculate the slope index (SI) for the brain region comparisons described above. The SI determined if a brain region is allometric ($\beta \neq 1$), meaning that sensory brain region (y)/central brain (x) would change with size. We also used a Wald Test to calculate the common shift (H^0 = equal axis among phenotypes), for any shift along the x axis. Finally, we calculated how much larger a sensory region (y) is compared to the central brain (y), by using a grade shift index (GSI) to compare phenotypes (i.e., $H^0 = \alpha_{\,males} = \alpha_{\,reproductives} = \alpha_{workers}$). The GSI reflected changes in intercept α (elevation) with no changes in the slope β. This method facilitates pairwise volumetric comparisons between phenotypes (i.e., $e^{\,\alpha\ males-\ \alpha\ reproductives}$), by implementing a Wald test. For example, if GSI > 1, males had larger volume of a brain region compared to reproductives, and if GSI < 1 the relationship would be inverse. We specify the direction of change for each of the analyzed categories in "Results" section and **Supplementary Tables 1**, **2**. Lastly, we also ran a Kruskal-Wallis test with subsequent pairwise comparisons to determine corpora allata growth across castes and to test the effect of both parasite and host sex.

RESULTS

Investment in Sensory Regions by Caste and Sex

All brain regions, except for the central complex showed a common slope, but had differences in the GSI, common shift and/or SI depending on the specific region (**Figure 2** and **Supplementary Table 1**). Males and reproductive females had proportionally larger pooled sensory regions compared to workers (GSI = 1.056, P = 0.01 and GSI = 1.036, P = 0.006, respectively, **Figure 2C**). Males had proportionally smaller antennal lobes when compared to reproductive females (GSI = 0.87, $P < 0.001$), as an effect of both changes in elevation and a common shift (**Figure 2D** and **Supplementary Table 1**). Males also had smaller antennal lobe volume than workers (GSI = 0.926, P = 0.002, **Figure 2D**). Males had larger optic lobes than reproductive females (GSI = 1.064, P = 0.001) and workers (GSI = 1.103, $P < 0.001$, **Figure 2E** and **Supplementary Table 1**). Within females, reproductives had larger antennal lobes (GSI = 1.057, P = 0.002, **Figure 2D**) and calyces compared to workers (GSI = 1.042, P = 0.003, **Figure 2F**). Reproductives had increased optic lobe volume compared to workers (GSI = 1.037, P = 0.02, **Figure 2E**). Finally, workers showed an isometric increase in the central complex (P = 0.052), in contrast to a hypoallometric reduction of this navigational brain region in reproductive females and males (**Figure 2I**).

Investment in Sensory Regions by Parasitized and Non-parasitized Wasps

Workers parasitized by one female or one male *X. vesparum* showed no differences in allocation of most sensory brain regions, compared to non-parasitized workers (**Figures 3A, C, G**). Indeed, non-parasitized workers shared a common slope with workers with a female or a male *X. vesparum*, and no volumetric differences in the antennal lobes (**Figure 3D**) or the optic lobes (**Figure 3E** and **Supplementary Table 1**). However, we did find a change in the slope index of the whole brain in workers parasitized by a female, compared to non-parasitized workers or those parasitized by a male ($P < 0.001$, **Figure 3B** and **Supplementary Table 1**). Workers parasitized by one female had an isometric pattern, resulting in larger calyces (P = 0.031, **Figure 3F**) and collars (P = 0.045, **Figure 3H**), than non-parasitized workers and those parasitized by one male. Lastly,

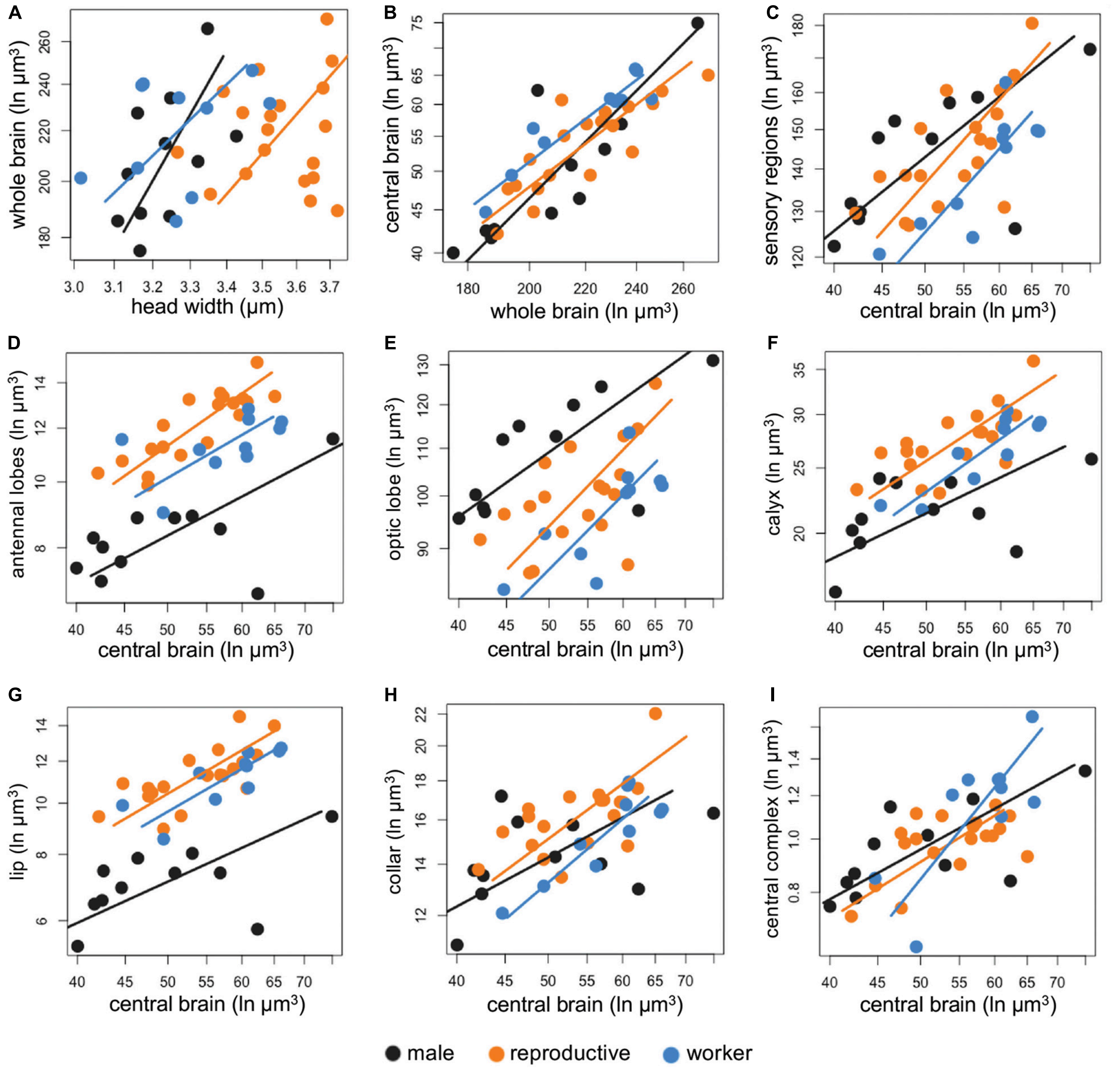

FIGURE 2 | (A–I) Comparison of the investment in sensory regions by sex and caste. The volume of each brain region was log-transformed, and each dot represents one individual. The males are depicted in black, reproductives (gynes and foundresses) in dark orange and workers in blue. The corresponding colored lines represent the slope for each category, and each dot represents an individual. Given that most comparisons shared a common slope, see **Supplementary Table 1** for full Standardized major axis (SMA) results.

workers with one male parasite had a hypoallometric reduction of the central complex in comparison to non-parasitized workers and those parasitized by a female ($P = 0.027$, **Figure 3I**).

In contrast, parasitized and non-parasitized males showed differential allocation toward specific brain regions. They shared a common slope and differences in grade shifts for the following brain regions: whole brain, antennal brain, lip, and central complex (**Supplementary Table 2**). Parasitized males had a proportionally smaller whole brains than non-parasitized males (GSI = 1.15, $P < 0.001$, **Supplementary Table 2** and **Figure 4A**). However, due to a common shift along the main slope axis, parasitized males had proportionally large antennal lobes ($P = 0.01$, **Figure 4D**), lip ($P = 0.001$, **Figure 4G**) and central complex ($P < 0.001$, **Figure 4I**) compared to non-parasitized males (**Supplementary Table 1**). In contrast, parasitized males showed a disproportionately reduced volume of the central brain ($P = 0.02$, **Figure 4B**), but disproportionately large volume of pooled sensory regions ($P = 0.03$ **Figure 4C**), optic lobes ($P = 0.03$,

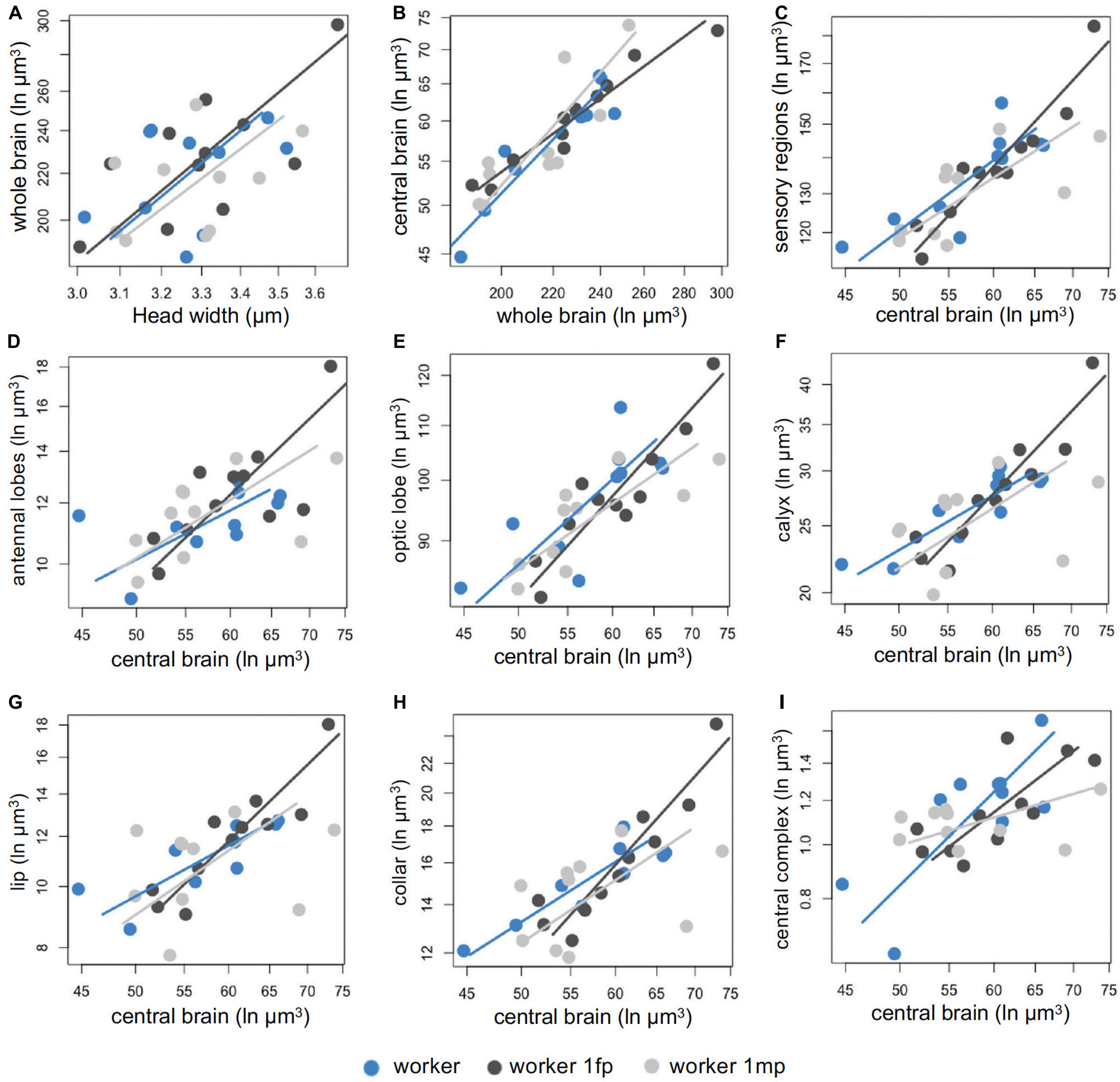

FIGURE 3 | (A–I) Comparison of the investment in sensory regions by parasitized and non-parasitized workers. Categories are depicted as non-parasitized workers (blue), workers parasitized by one female *X. vesparum* (dark gray) and workers parasitized by one male *X. vesparum* (light gray). The corresponding color-coded line represents the slope for each category and each dot represents an individual. Some comparisons shared a common slope, see **Supplementary Table 1** for full statistical results.

Figure 4D), calyces ($P = 0.04$, **Figure 4F**), and collar ($P = 0.02$, **Figure 4H**) compared to non-parasitized males (**Figure 4** and **Supplementary Table 2**).

Corpora Allata Development According to Sex, Caste, and Parasitism

The corpora allata were significantly smaller in all males compared to females ($\chi^2 = 46.86$, df = 6, $P < 0.001$, **Figure 5**). Although not significant, foundresses showed the expected trend toward large corpora allata compared to gynes ($Z = 2.02$, $P = 0.07$) and workers ($Z = 0.79$, $P = 0.06$). *Post hoc* pairwise tests showed no significant differences in workers parasitized by one female ($Z = 1.62$, $P = 0.1$) and by one male ($Z = 4.41$, $P = 0.5$) compared to non-parasitized workers. Gland size also did not differ according to parasite sex ($Z = -0.84$, $P = 0.39$), or between parasitized and non-parasitized males ($Z = -0.02$, $P = 0.98$).

DISCUSSION

We provide several lines of evidence supporting the focal hypothesis that brain plasticity facilitates differential sensory needs and life histories within the same species. First,

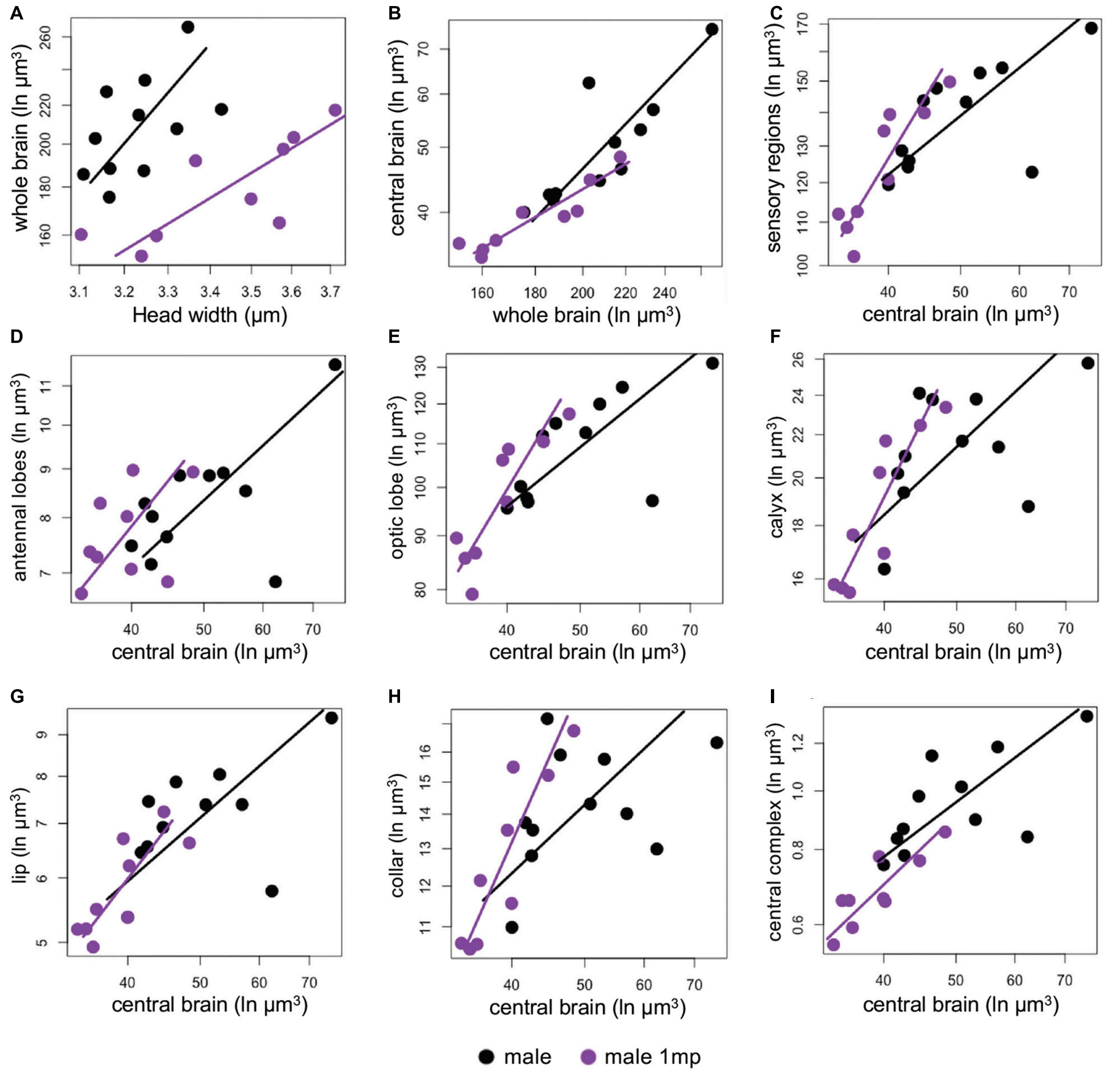

FIGURE 4 | (A–I) Comparison of the investment in sensory regions by parasitized (purple) and non-parasitized males (black). SMA fits are log-transformed per categories with the lines based on intercepts and slopes (purple for parasitized and black for non-parasitized males). Most volumetric comparisons did not share a common slope, see **Supplementary Table 2** for full SMA tests.

reproductive females also had larger calyces compared to worker females, reflecting sensory needs associated with division of labor. Second, males and females showed a consistent and significant differential investment in volume of the optic and antennal lobes. This pattern implies a life history-based plasticity of otherwise genetically shared backgrounds in *Polistes dominula*. Furthermore, we provide novel evidence for the effect of the *Xenos vesparum* parasite in neural investment by female and male hosts. Contrary to our prediction, non-parasitized and parasitized workers show moderate volumetric differences in brain sensory regions, while parasitized males showed a more drastic effect in allocation of neural tissue compared to non-parasitized males. Overall, our results are consistent with differential investment in brain regions being advantageous across social wasp species (O′Donnell et al., 2011).

The observed differential investment in sensory brain regions reflect the distinct life cycles of *P. dominula* males and females, similarly to previous studies in bees and ants (Van Praagh et al., 1980; Arnold et al., 1985; Menzel et al., 1991; Gronenberg, 2008; Mysore et al., 2009). Together, these findings suggest that in social Hymenoptera, male sexual behavior is a fundamental driver of neural organization (Beani et al., 2014). Male *P. dominula* leave their nest within a few days after emergence and gather at distinct leks to increase their mating opportunities (Beani, 1996). When attempting to mate, males can visually distinguish between females and competing males, and between workers and gynes

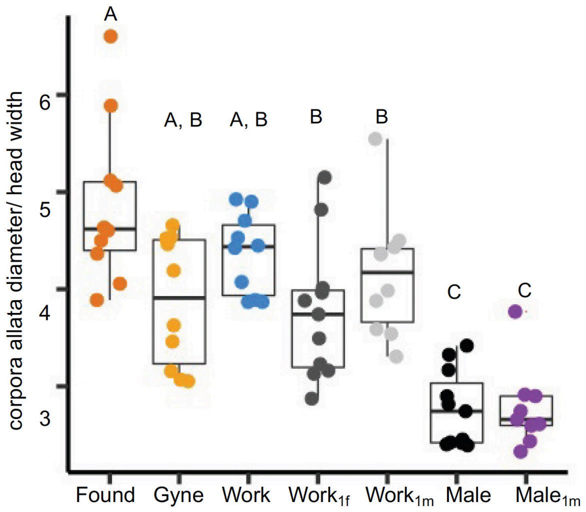

FIGURE 5 | Corpora allata size in reproductive females, workers, males, parasitized workers, and parasitized males. The cross-sectional area of the corpora allata was calculated by measuring its diameter in microns and normalized with head size. Wasp categories are color coded: found (foundresses), gynes, work (workers), work$_{1fm}$ (workers with 1 female parasite), work$_{1m}$ (worker with 1 male parasite), males and male$_{1m}$ (male with 1 male parasite). Each boxplot shows the median, 25th and 75th percentiles. The whiskers show the 5th and 95th percentiles.

(Cappa et al., 2013; de Souza et al., 2017; da Silva et al., 2021). Therefore, larger optic lobes may facilitate detection and discrimination between potential mates or male intruders in their defended territories (Beani et al., 2014). Males have smaller antennal lobes, which is likely due to experiencing less complex olfactory stimuli, as they do not engage in frequent chemically-based social interactions in the colony. In contrast, reproductive females have proportionally larger antennal lobes, lips and collars, which is consistent with other studies that show sensory needs associated with division of labor, interactions among nestmates, learning and memory (Gronenberg et al., 1996; Ehmer and Hoy, 2000; O′Donnell et al., 2011; Mora-Kepfer, 2014; Jernigan et al., 2021; Mertes et al., 2021; Rozanski et al., 2021; Uy et al., 2021). Thus, the social environment of female wasps has a wider range of chemical and sensory processing cues compared to males (Beani et al., 2014).

Within females, reproductives had proportionally larger calyces than workers, which coincides with division of labor in these social wasps (O′Donnell et al., 2007). Foundresses consistently engage in social interactions both within the colony and as gynes during winter aggregations, utilizing visual and chemical cues toward recognition (Dani et al., 2001; Cini et al., 2019). In contrast, most workers spend less time interacting with foundresses and brood on the nest, and allocate more time performing tasks such as foraging for prey and building material (Gamboa et al., 1978). Our results are similar to studies in *P. dominula* (Ehmer et al., 2001), *P. instabilis* (Molina and O’Donnell, 2007), and *Mischocyttarus mastigophorus* (O′Donnell et al., 2007) that correlate large calyces with social dominance. Contrastingly, in ants, honey bees, and *Polybia* paper wasps, subordinate forager workers have large calyces (Withers et al., 1993; Gronenberg et al., 1996; O′Donnell et al., 2004). However, these social insects form large colonies, and show specialized division of labor and age polyethism. Specifically, workers transition from tasks inside the nest to more sensory-demanding tasks outside the nest such as navigation and learning landmarks, which supports the pattern of large calyces (Gronenberg and Riveros, 2009; O′Donnell et al., 2011; Cabirol et al., 2018). Finally, differences in nutrition may also influence allocation of brain tissue between workers and foragers. In *P. metricus*, lower nutrition is associated with higher foraging and brain gene expression in workers (Toth et al., 2009; Daugherty et al., 2011). Similarly, in the primitively-eusocial bee *Augochlorella aurata*, queens have larger mushroom bodies than workers, due to increased nutrition as larvae (Pahlke et al., 2019).

Contrary to our expectations, parasites have small effects on the brain architecture of workers and did not elicit a significant reduction in the corpora allata. Male parasites induced a reduction of the central complex, compared to non-parasitized workers or workers parasitized by one female. This difference in the central complex, which is mainly implicated in spatial navigation, is not consistent with the lack of differences in behavior between workers infected by the two sexes. Noticeably, workers parasitized by one *X. vesparum* female showed larger calyces than non-parasitized workers and those parasitized by one male. Interestingly, workers parasitized by one female enter diapause and resemble the behavioral and physiological phenotype of overwintering gynes, while workers parasitized by a male die at the end of summer like non-parasitized ones (Beani et al., 2021). Thus, it is likely that the female parasite should minimize cognitive impairment of its worker host.

In contrast, male parasites had a more drastic effect in the brain architecture of *P. dominula* males, which are parasitized less frequently than females. Parasitized males had significantly smaller whole brains and central brains than non-parasitized males. They also showed a significant increase in the volume of several sensory brain regions, including the antennal and optic lobes, and two substructures of the calyx: lip and collar. Remarkably, neuroendocrine manipulation does not seem to occur in parasitized males, as they develop their corpora allata, testes, seminal vesicles and accessory glands and attempt to mate (Cappa et al., 2014; Beani et al., 2017). The inability to castrate the male may likely result in brain manipulation instead. Given that the brain is an expensive tissue to produce (Niven and Laughlin, 2008; Keesey et al., 2020), the parasite may reallocate energy and resources to develop inside the host, resulting in the observed small brains.

Notably, only a few studies have tested for neuroanatomical changes induced by parasites that do not directly infect in the brain, but instead lodge inside insect body cavities. The parasitic fungus *Ophiocordyceps* manipulates the behavior of their ant host, but does not induce structural changes in the brain (Hughes et al., 2011). Instead, the fungal hyphae surround muscle fibers (Hughes et al., 2011; Fredericksen et al., 2017). The hairworm *Paragordius tricuspidatus* induces neurogenesis in the mushroom bodies of its cricket host, before manipulating it to jump into the water to continue the parasite’s lifecycle

(Thomas et al., 2003). Infected crickets also showed differential expression of proteins in the head (Biron et al., 2006). Together with our findings, these results suggest that different parasites likely hijack distinct neural mechanisms to control the behavior of their insect hosts (Hughes and Libersat, 2018).

Overall, our results demonstrate that brain plasticity is associated with sensory needs in males and within female castes of *P. dominula*, but that parasitic manipulation can also drive differential investment of brain regions depending on both host and parasite sex. Intriguingly, workers infected with a female parasite show a strong manipulation effect of the parasite on caste determination, lipid storage, and prolonged lifespan in parasitized females that act as their main host (Beani et al., 2021), but more dampened effects on allocation of brain tissue. In turn, the reproductive apparatus and behavior of parasitized males are essentially unaffected, but they experience stronger volumetric changes in brain regions. Previous work has shown that *X. vesparum* drives gene expression changes of workers toward a gyne-like pattern; thus, the parasite is manipulating the transcriptomic plasticity of the caste system (Geffre et al., 2017). Parasitized females also show low levels of haemolymphatic protein and juvenile hormone compared to non-parasitized females, but with no difference between non- parasitized and parasitized males (Strambi and Strambi, 1973; Strambi et al., 1982). Our study shows that strepsipteran do not drive evident neuroanatomical changes in their females hosts, suggesting that this parasite may be relying on other manipulation mechanisms (Libersat et al., 2018).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LB and FC collected the wasp and parasite field samples. KG performed histological preparation of specimens and collected volumetric data. FU analyzed the data. KG, LB, FC, and FU contributed to the first draft of the manuscript. All authors provided input to concept and design of this project, data interpretation, along with reviewing and editing the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.803437/full#supplementary-material

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Socioecology and Evolutionary Neurobiology of Predatory Ants

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Diet and social complexity are hypothesized to drive the evolution of the neuroarchitecture of the brain, but the relative impacts of foraging ecology and social organization have not been fully disentangled. Predatory ant species encompass generalists as well as specialists on remarkably narrow ranges of arthropod prey, and vary in strategy from solitary hunting to group raiding. Dietary differences and variation in individual or group predation appear to be correlated with the use of vision for navigation by solitary huntresses, the predominance of chemical signaling to organize group predation, and the structure, biomechanics, and sensorimotor control of the mandibles, and likely gustatory sensilla. Predatory ants provide the opportunity to separate the relative roles of diet and colony size and brain structure, and offer diverse novel systems to understand adaptive brain mosaicism and the neuronal regulation of predatory behavior. Here we discuss the socioecology of predatory ants and its influence on neuroanatomy.

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THE NEUROBIOLOGY OF PREDATION

Neuroethological and molecular studies of visual, olfactory, auditory, pheromonal, electrical, and mechanoreceptive sensory systems have identified circuitry underpinning predatory behavior in diverse animal clades (Sillar et al., 2016). Star-nosed moles (Catania, 2011), electric fishes (Sukhum et al., 2018), and bats (Genzel et al., 2018) are renowned models. Predator sensory systems generally reflect foraging ecology. Many predatory insects, for example, have large eyes to detect and pursue moving prey through interceptive or ambush hunting strategies. Optic lobe neurons tuned to the motion of small moving objects regulate predatory behavior (Wardill et al., 2015, 2017; Fabian et al., 2018; Nicholas et al., 2018; Nityananda et al., 2018; Supple et al., 2020). Predators may also exhibit morphological and anatomical adaptations to prey type (Martinez et al., 2018) and/or group hunting strategies (Lang and Farine, 2017; Bastos et al., 2021). Predatory behavior in ants evolved independently multiple times in virtually all major subfamilies, including basal clades (Rabeling et al., 2008; Ward, 2014) and most predatory genera are sociobiologically and ecologically diverse (Keller and Peeters, 2020). Predatory ants often show striking differences in diet—usually linked to differences in mandible morphology, biomechanics, motor, olfactory and likely gustatory systems—and social organization. An evolutionary approach can integrate studies of foraging ecology, social structure, morphological evolution, neuroanatomy, and neurophysiology.

MANDIBULAR MORPHOLOGY, SENSORY BIOLOGY, AND MOTOR CONTROL IN PREDATORY ANTS

The morphology of the mandibles—the primary appendages ants use like tools to manipulate their environment and capture prey—varies widely across taxa. Most ants, including some extinct and extant predatory species, have triangular, shovel-shaped mandibles, but those of many predatory species have extreme morphologies (**Figure 1**) and biomechanical adaptations to specialized diets and prey-capture strategies (Brown and Wilson, 1959; Masuko, 1993, 2019, 2020; Dejean, 1997; Dejean and Dejean, 1998; Rabeling et al., 2012; Schmidt and Shattuck, 2014; Probst et al., 2015; Barden et al., 2020; Keller and Peeters, 2020). Predatory ant mandibles have associated neuronal mechanisms that, in some cases, control remarkably rapid closure. In trap-jaw ants, the mandibles can be cocked back (like a mousetrap) and then used to strike prey in as little as 0.33 ms. Bite force and speed of depend on mandibular muscle biomechanics and properties of the motor neurons that innervate them (Gronenberg, 1996; Just and Gronenberg, 1999). Trap-jaw mandibles typically have sensory trigger hairs that respond to prey contact with a high-frequency burst of action potentials and project into the subesophageal zone (SEZ; Gronenberg et al., 1998a,b; Gronenberg and Riveros, 2009), a brain compartment involved in sensorimotor control of the mandibles, mouthparts, feeding behaviors, and gustation. The motor and chemosensory information transduced by sensilla is eventually processed by the mushroom bodies (MBs) a higher-order brain compartment strongly linked to learning, memory, and behavioral plasticity (Fahrbach, 2006; Gronenberg, 2008; Wright, 2016). Gronenberg et al. (1993) first described how the sensory-motor reflex of the trap-jaw strike of predatory *Odontomachus* workers is controlled by mechanoreceptor trigger hairs with large, rapidly conducting axons in what is likely a monosynaptic connection with motor neurons. Comparative analyses of trap-jaw mechanisms, which have evolved independently in multiple ant genera, reveal convergent biomechanical and neurobiological traits (Gronenberg, 1996; Larabee et al., 2017). Mandible closure velocity in trap-jaw ants appears to differ among species due to phylogeny, physiology, and prey specialization (Larabee et al., 2017, 2018; Gibson et al., 2018). The study of mandible morphology and neurobiology (motor control and sensory capabilities) of predatory ants may thus shed light on the relationships of diet, prey recognition, hunting and prey-capture strategies, and brain organization. Additionally, brain compartments associated with feeding (e.g., SEZ) may scale allometrically with prey-catching strategies, prey specialization, and colony size (Kamhi et al., 2017; Miroschnikow et al., 2020).

DIET, SOCIALITY, AND BRAIN EVOLUTION

Ecological brain theory hypothesizes that the behavioral and/or cognitive challenges of locating and processing food play a key role in brain evolution (Harvey et al., 1980; Goldman-Huertas et al., 2015; DeCasien et al., 2017; Lihoreau et al., 2019; Simons and Tibbetts, 2019). A high-quality (e.g., frugivorous) diet correlates with large brain size and expanded olfactory or visual systems in primates (Dunbar and Shultz, 2017; DeCasien and Higham, 2019). Social brain theory hypothesizes that brain size increases with group size and social complexity, due to cognitive challenges associated with increased conflict and cooperation (Dunbar and Shultz, 2017). Vertebrate societies are characterized by reproductive competition and social bonding, but eusocial insect workers are generally sterile. The applicability of social brain theory as developed for vertebrates to eusocial insects has thus been questioned (Lihoreau et al., 2012; Farris, 2016). Here we use the term social complexity as a working concept consistent with Anderson and McShea (2001): socially complex ants have large colony size, worker polymorphism and division of labor, and collective foraging strategies. Dornhaus et al. (2012) further discuss how collective organization may scale with colony size. Empirical studies of eusocial insect brain evolution indicate increased social complexity may increase *or* decrease worker brain size in larger colonies depending on reproductive conflict and division of labor (Jaffe and Perez, 1989; Wehner et al., 2007; Riveros et al., 2012; Muscedere et al., 2014; O'Donnell et al., 2015, 2018; Kamhi et al., 2016; Godfrey and Gronenberg, 2019; DeSilva et al., 2021). Brain structure is also known to change with worker age (Seid et al., 2008; Muscedere and Traniello, 2012) or task specializations that may develop in large colonies (Amador-Vargas et al., 2015). Variation in diet, social organization, and behavioral polyphenisms in insects may be underpinned by neuroanatomical differentiation. Brain size in insects correlates with life history and diet (Farris and Roberts, 2005; Farris, 2008; Bouchebti and Arganda, 2020) and an increase in MB size, potentially supporting enhanced foraging-related navigation and memory (Sayol et al., 2020). At a cellular scale, the density of MB synaptic complexes (microglomeruli, MG) correlates with age, subcaste, task specialization or increase in behavioral repertoire (Groh and Rössler, 2011; Groh et al., 2014; Kamhi et al., 2017; Gordon and Traniello, 2018; Gordon et al., 2018), or requirements for higher-order processing involved in learning and memory (Li et al., 2017). Memory may be associated with a transient increase in MG density (Falibene et al., 2015). These latter studies are among the few suggesting a link between diet, social behavior, and brain evolution in insects. The relationship between diet and MB evolution remains poorly understood.

PREDATORY ANTS AS MODELS OF BRAIN EVOLUTION

Predatory behavior in ants evolved independently multiple times in virtually all major subfamilies, including basal clades. Predatory ants are widely distributed and sociobiologically and ecologically diverse. Workers are active predators, and species show striking differences in prey specialization, dietary breadth, and colony size (range from <10 workers [*Thaumatomyrmex* spp.] to 20 million [*Dorylus wilverthi*]), worker polymorphism, and division of labor. These ants thus have the potential to offer new insights into the relationship between social organization, diet, brain size, and mosaic structure. Army ants (Subfamily Dorylinae) are mass-foraging generalist or specialist predators that may form huge colonies of morphologically and behaviorally specialized workers (Kronauer, 2020; McKenzie et al., 2021). Predatory poneroid ants hunt alone or in groups and differ in diet and social complexity (Peeters, 1997; Ward, 2014; Hanisch et al., 2020). Solitary huntresses in some species broadly attack invertebrates whereas others specialize on termites, an energetically valuable clumped and sessile resource (**Figure 1**). The shift from randomly distributed prey to clumped prey involves changes in foraging behavior, resulting

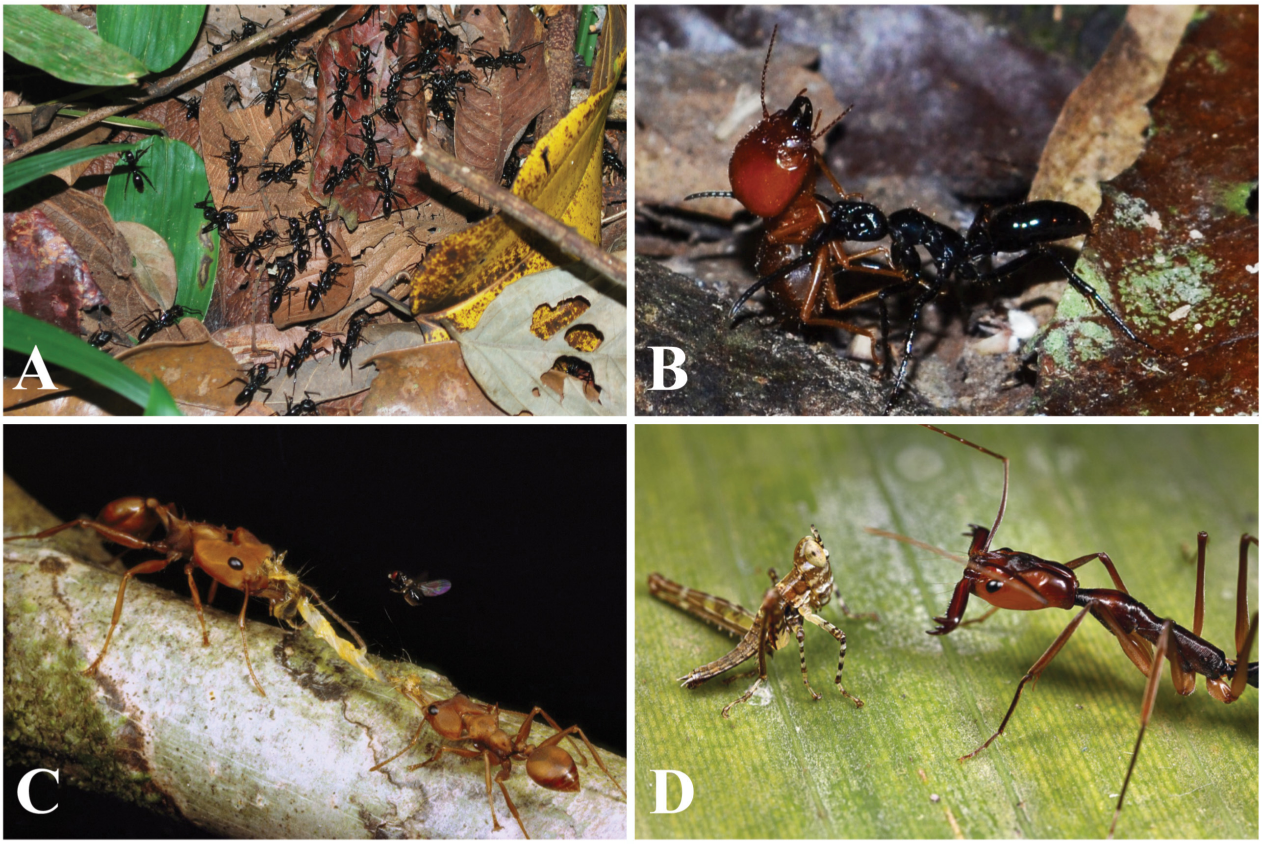

FIGURE 1 | Predatory behavior in ants. **(A)** Group foraging in *Neoponera commutata*, a specialized predator of termites, and **(B)** prey retrieval. **(C)** *Daceton armigerum* workers retrieving prey. **(D)** *Odontomachus*, a trap-jaw ant.

in an apparent decrease in use of vision (e.g., ommatidia size and/or number) for navigation by solitary workers to chemical signaling to organize group predation (Hölldobler and Traniello, 1980; Mill, 1984; Dejean and Lachaud, 2011; Jelley and Barden, 2021; Sosiak and Barden, 2021). These differences in hunting and prey-capture strategies, as well as the involvement of different sensory modalities in prey localization (Masuko, 1990; Gronenberg and Tautz, 1994; De la Mora et al., 2008), are associated with changes in behavioral demands for prey recognition, foraging communication, and foraging-task specialization (Schmidt and Overal, 2009) that will be reflected in volumetric changes in functionally specialized brain compartments. Other socioecological traits (activity pattern, nesting and foraging habits, foraging range, and prey distribution) are associated with morphological adaptations such as eye and antenna size, and sensilla type and density, and in turn linked with prey selection, diet, and brain mosaicism (Menzi, 1987; Polidori et al., 2012; Narendra et al., 2013; Ramirez-Esquivel et al., 2014; Bulova et al., 2016; Wittwer et al., 2017; Heinze et al., 2018; McKenzie et al., 2021). Prey olfactory detection and discrimination likely depend on the diversity of sensillae and receptors, and their neuronal projections into individual antennal lobe glomeruli that vary in size and number (Couto et al., 2005; van der Woude and Smid, 2016). These characteristics make predatory ants useful models to understand how dietary shifts may have shaped colony size and complexity, individual and group behavior, and brain and sensory system structure.

COMPARATIVE AND PHYLOGENETIC ANALYSES

Species that vary strongly in diet, predatory strategy, and social complexity can be compared to identify selective influences on brain mosaicism and synaptic architecture. For example, *Neoponera laevigata* is a specialized group-predator of termites: workers are polymorphic, colonies are relatively large (∼1,500) and nomadic (Downing, 1978). In contrast, workers of the sister species *N. apicalis* are monomorphic generalist predators that forage solitarily and form small colonies (∼200 workers; Schmidt and Shattuck, 2014). Other species that differ socioecologically include *N. villosa* (large colonies, generalist diet) and *Leptogenys langi* and *Stigmatomma pallipes* (small colonies, specialist diet). The influences of social organization and diet on brain compartment scaling and MG densities can thus be distinguished given that species have small or large colony size and generalist or specialist diets. Brain compartment volume variation of the [MBs], the optic [OL], the antennal lobes (AL; visual and olfactory information processing, respectively), and the SEZ can then be assessed to test the following hypotheses:

- If colony size and associated increases in social interactions, rather than diet, drive brain evolution, then species with large colonies with either generalist or specialist diets are hypothesized to have allometrically large MBs to process social information.
- If diet has a primary influence on brain evolution, then workers of prey-generalist species with both small and large colonies will have large MBs compared to specialist species (higher demands for navigational skills involving learning and memory [MB elaboration and increased MG density] in prey-generalist species; prey-specialist species depend on chemical signaling during foraging).
- Species with generalist diets that vary in behavioral and/or cognitive demands for prey recognition and navigation are predicted to have similar compartmental scaling in the OLs and ALs, and MG densities in large and small colonies (macroscopic and synaptic neuroanatomy are independent of colony size).
- Prey-specialist species will have reduced OL and increased AL size and a decrease in MG density in association with relative demands for processing visual (MB collar) and olfactory (MB lip) information, respectively.
- Prey-generalist species will have larger SEZ and a higher diversity and size of AL glomeruli (need to discriminate among prey).
- If the interaction of diet and colony size influence brain size evolution, then workers of prey-generalist species with large colonies will have allometrically large MBs (higher demands on sensory and behavioral functions).
- Neuroanatomical scaling and socioecology can be mapped phylogenetically to identify patterns of brain evolution. Detailed cellular analyses can be informed by and benefit from this broad analysis.

CONCLUSION

Our understanding of the relationship between diet, sociality, brain size, compartmental scaling, synaptic architecture, and other neuroethological underpinnings of behavior can benefit from studies of predatory ants. Integrated sociobiological, ecological, morphological, neurobiological, transcriptomic, and genomic research is needed to understand the evolution of individual and group predatory strategies. Brain evolution can thus focus on the behavioral ecology of predation. Studies of morphological evolution can be integrated with sensory biology and motor control of the mandibles as well as the scaling of functionally specialized brain centers. The characterization of olfactory sensilla and their receptors on the mandibles, other mouthparts, and antennae, and investigation of AL glomeruli size and distribution are needed to understand mechanisms of gustation and their relationship with diet preference, prey identification, and prey-capture strategy. Additionally, we can infer whether gains and losses in neuroarchitecture are significantly associated with clades bearing particular individual worker and social traits and dietary habits.

AUTHOR CONTRIBUTIONS

FA, MM, and JT: conceived, wrote, and edited the manuscript. JT: secured funding. All authors contributed to editing the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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Statistical Atlases and Automatic Labeling Strategies to Accelerate the Analysis of Social Insect Brain Evolution

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Current methods used to quantify brain size and compartmental scaling relationships in studies of social insect brain evolution involve manual annotations of images from histological samples, confocal microscopy or other sources. This process is susceptible to human bias and error and requires time-consuming effort by expert annotators. Standardized brain atlases, constructed through 3D registration and automatic segmentation, surmount these issues while increasing throughput to robustly sample diverse morphological and behavioral phenotypes. Here we design and evaluate three strategies to construct statistical brain atlases, or templates, using ants as a model taxon. The first technique creates a template by registering multiple brains of the same species. Brain regions are manually annotated on the template, and the labels are transformed back to each individual brain to obtain an automatic annotation, or to any other brain aligned with the template. The second strategy also creates a template from multiple brain images but obtains labels as a consensus from multiple manual annotations of individual brains comprising the template. The third technique is based on a template comprising brains from multiple species and the consensus of their labels. We used volume similarity as a metric to evaluate the automatic segmentation produced by each method against the inter- and intra-individual variability of human expert annotators. We found that automatic and manual methods are equivalent in volume accuracy, making the template technique an extraordinary tool to accelerate data collection and reduce human bias in the study of the evolutionary neurobiology of ants and other insects.

Keywords: standardized brain atlases, computational neuroimaging, evolutionary neurobiology, neuroethology, social brain evolution, Neuroanatomy, Ant brains

INTRODUCTION

Our understanding of pattern and process in brain evolution in group-living animals benefits from sampling phylogenetically diverse species. Ants and other eusocial insects (primarily wasps, bees, and termites) have become important models to explore what is broadly conceptualized as "social brain evolution" (Dunbar, 1998; Lihoreau et al., 2012, 2019; Godfrey and Gronenberg, 2019; Muratore and Traniello, 2020; Coto and Traniello, 2021). Eusocial insects have exceptional reproductive and ergonomic polyphenisms associated with division of labor and highly cooperative behavior, and thus offer multiple opportunities and a rich array of species to examine how reproductive competence, sterility, and morphological and behavioral differentiation impact social roles and neuroarchitecture. Workers show extraordinary behavior as individuals as well as members of groups that act collectively, and individuals are so interdependent that the colony is considered to be a "superorganism" (Hölldobler and Wilson, 2009). The brains of colony members have evolved to respond as individuals but also as decision-making groups to cope socially with the environment and its challenges, as well as facilitate communication and coordinate foraging, defense, and nest construction and regulate task performance and nestmate recognition. Important questions integrating insect sociobiology and evolutionary neurobiology concern how selection may favor either an increase or reduction in brain size and structure (Wehner et al., 2007; Muscedere and Traniello, 2012; Riveros et al., 2012; O'Donnell et al., 2018; Arganda et al., 2020; DeSilva et al., 2021).

Ant brains and those of other insects can be adaptive allometric mosaics composed of functionally specialized brain compartment allometries. Neuropils are involved in primary sensory processing (e.g., the antennal, optic lobes, subesophageal zone), motor control and navigation (the central complex and subesophageal zone), and multi-sensorial higher-order processing and integration, learning and memory (the mushroom bodies) (Strausfeld, 2012). Immunohistochemistry, confocal microscopy, and other techniques are commonly used to image brains and neuropil volumes are quantified using image analysis software to examine brain structure within and across insect species. Methods to calculate neuropil volumes require allocating significant effort to manually annotate brain compartments and subregions because an anatomical label must be assigned to every pixel or voxel in 2D and 3D images, respectively (**Figure 1**). This technique of recording neuroanatomical data is both time consuming and susceptible to human bias and error.

Technical problems associated with imaging ant brains can be reduced by using methodologies developed to study the human brain (Talairach and Tournoux, 1988). These techniques usually combine images from multiple brains into a single reference brain or *template* (**Figure 2**). This method has been applied in studies on honey bees (e.g., Rybak, 2012), flies (e.g., Rein et al., 2002; Costa et al., 2016; Arganda-Carreras et al., 2018), and other insects (e.g., Kurylas et al., 2008; Menzel, 2012; el Jundi and Heinze, 2020). The use of several brain images to build a template avoids potential biases arising during tissue fixation and imaging, and accounts for the natural variability among samples, allows a statistical representation of the brain of a species or worker phenotype. This type of template, as opposed to a reference brain derived from a single individual, is called a "group-wise template." Because combining all samples in a single brain representation requires transforming them onto the same reference space, templates allow normalizing information from brains that might have been imaged under different conditions. In addition, group-wise templates are usually associated to annotations (labels) of brain subcompartments. These labels of the template are used to automatically segment (annotate or label) these sub-compartments in new samples, by registering them against the template, which consist of transforming them to be in the same reference space as the template (e.g., Arganda-Carreras et al., 2018). An alternative to his strategy is that of Rybak (2012), where a template brain is created in a similar way to ours, but individual brains are first labeled using a statistical shape model and then registered against the template using the label volumes instead of the gray-value ones. This approach has the advantage of a label-oriented registration, where each anatomical region can be treated independently. However, its performance may be too sensitive to the segmentation result obtained by the model, which should correctly estimate the sometimes very large shape diversity of the dataset.

Although template strategies have been widely applied in mammals (Talairach and Tournoux, 1988; Evans et al., 1994; Mazziotta et al., 1995; Chen et al., 2006; Dogdas et al., 2007; Shattuck et al., 2008; Yu et al., 2010), their implementation in insect research has been less frequent. It has been expanded from *Drosophila* (Rein et al., 2002; Jefferis et al., 2007; Cachero et al., 2010; Costa et al., 2016; Arganda-Carreras et al., 2018) to other insects only in the last decade (e.g., Menzel, 2012; Rybak, 2012; el Jundi and Heinze, 2020). The application of this methodology to research focusing on ants has occurred more slowly, probably because of their high diversity (∼15,000 species). In addition, intra-specific variability is an issue *per se*: workers may show greater variation in brain anatomy at the level of species and colony, thus constraining the image registration process needed to generate a template, which usually requires a minimum spatial overlap of positions between same sub-regions in the co-registered brain images. Another difficulty is that neuroanatomical studies performed on ants focus on more or less detailed brain subdivisions, creating different sets of compartmental anatomical labels (e.g., Muscedere and Traniello, 2012; Amador-Vargas et al., 2015; Bressan et al., 2015; O'Donnell et al., 2018; Gordon et al., 2019; Sheehan et al., 2019; Habenstein et al., 2020). Consequently, most studies describing ant brain organization have not aimed at building brain templates (e.g., Bressan et al., 2015; Habenstein et al., 2020).

Here, we describe and evaluate experimental strategies to generate brain templates in ants to promote standardized approaches for comparative neuroanatomical analysis. While finer descriptions of neuropil sub-compartments exist for ant brains (e.g., Bressan et al., 2015; Habenstein et al., 2020) and

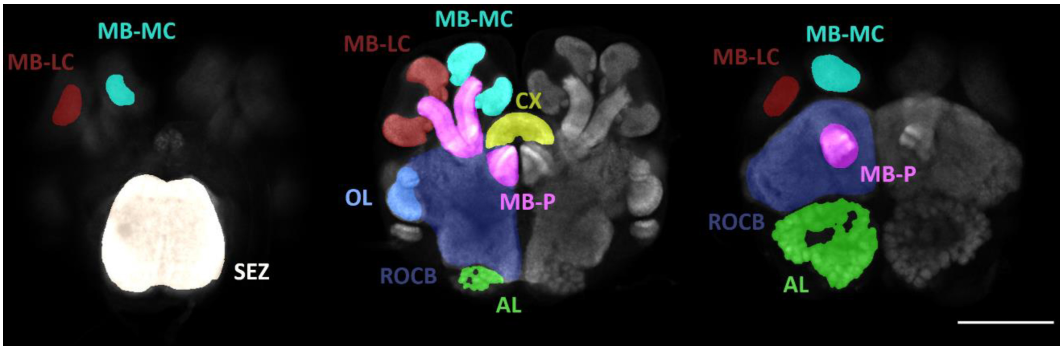

FIGURE 1 | Anatomy of a *P. spadonia* minor brain. MB-LC (mushroom body lateral calyx), MB-MC (mushroom body medial calyx), SEZ (subesophageal zone), OL (optic lobes), AL (antennal lobes), MB-P (mushroom body peduncle), CX (central complex), and ROCB (rest of the central brain). Scale bar = 100 um. Three brain slices have been selected to show all the subregions analyzed.

other social Hymenoptera (e.g., Brandt et al., 2005; Rybak, 2012; Groothuis et al., 2019), we focused this first approach on major neuropils (which are commonly used to explore neuroanatomical differences among species, castes, subcastes and experimentally manipulated individuals, e.g., Kamhi et al., 2016; Seid and Junge, 2016; Gordon et al., 2017; Grob et al., 2021). We recently applied state-of-the-art imaging techniques to generate templates using brains from a single or multiple ant species (Arganda-Carreras et al., 2017; Gordon et al., 2019). Using careful annotations by trained researchers as our standard, we evaluate template-based strategies to automatically segment ant brain confocal images, allowing more efficient and less biased volumetric data acquisition. We validate the template method by evaluating its application to workers of species in the ant genus *Pheidole*.

MATERIALS AND METHODS

We present three methods to produce and use templates for automatic segmentation (**Figure 2**). The first consists of building a template using confocal gray value whole brain images of a single species, and manually labeling brain compartments on the template (**Figure 2A**). This "direct label method" involves manually tracing a single anatomy (the one of the template) and automatically tracing other gray value brain images (by registration against the labeled template). The second "consensus label method" also uses a single-species template, but gray value brain images used to build the template contain manually annotated labels (**Figure 2B**). Then, these manual labels are used to create the final template labels. This method considers label values resulting from more than a single (potentially biased) tracing, and thus, it may be more accurate than the first method, at the expense of requiring more manual work. In this case, the method is only useful to trace new brains. The third possibility—the "multispecies template method"—is similar to the second but uses gray value brain images from several species (**Figure 2C**), thus enabling the expansion of species sampling. We next describe the ant brain dataset used, the methods to generate the different templates and labels, and how to evaluate the efficacies of the different methods.

Brain Anatomy Dataset

We imaged brains of minor workers of four species of the hyperdiverse ant genus *Pheidole* (*P. spadonia*, *P. rhea*, *P. tepicana,* and *P. obtusospinosa*). While *Pheidole* is typically characterized by complete dimorphism in the worker caste (small minor and large major [soldier] workers) and in some basal species (*P. rhea*) trimorphism, which includes a third, larger worker subcaste (super soldiers), we used only minors for proof of concept. Minors and majors are easily discriminated by body size and head allometry (Wilson, 2003).

Minor workers were decapitated and their brains were dissected from the head capsule in ice cold HEPES-buffered saline. Brains were fixed and immunohistochemically stained using SYNORF1 (a monoclonal *Drosophila* synapsin I antibody obtained from the Developmental Studies Hybridoma Bank, catalog 3C11) and secondarily stained using Alexa Fluor 488 for visualization of neuropil (slightly modified from Ott, 2008). Mounted in methyl salicylate, brains were imaged on an Olympus Fluoview BX50 laser (488 nm) scanning confocal microscope with a × 20 air objective (NA = 0.5) at a resolution of ~0.7 × 0.7 × 5μm/voxel, producing gray images of 16 bits (in TIFF format). We imaged 10 brains from *P. spadonia*, and three each of *P. rhea*, *P. tepicana*, and *P. obtusospinosa* minor workers. Each brain image was manually labeled as described in the "Manual labeling of original brain images and template" section below.

Standard Brain Image Method: Image Registration and Template Generation

Templates were built in a diffeomorphic space[1] as an average-shape brain ("Template," **Figure 2**). The diffeomorphic space allows for smooth invertible transformations from one anatomy to another (T^1 and T^{-1}, **Figure 2**). Our methodology is based

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[1] **Diffeomorphism:** differentiable transform that allows mapping the coordinates of one image onto the coordinates of another image in a smooth and invertible way.

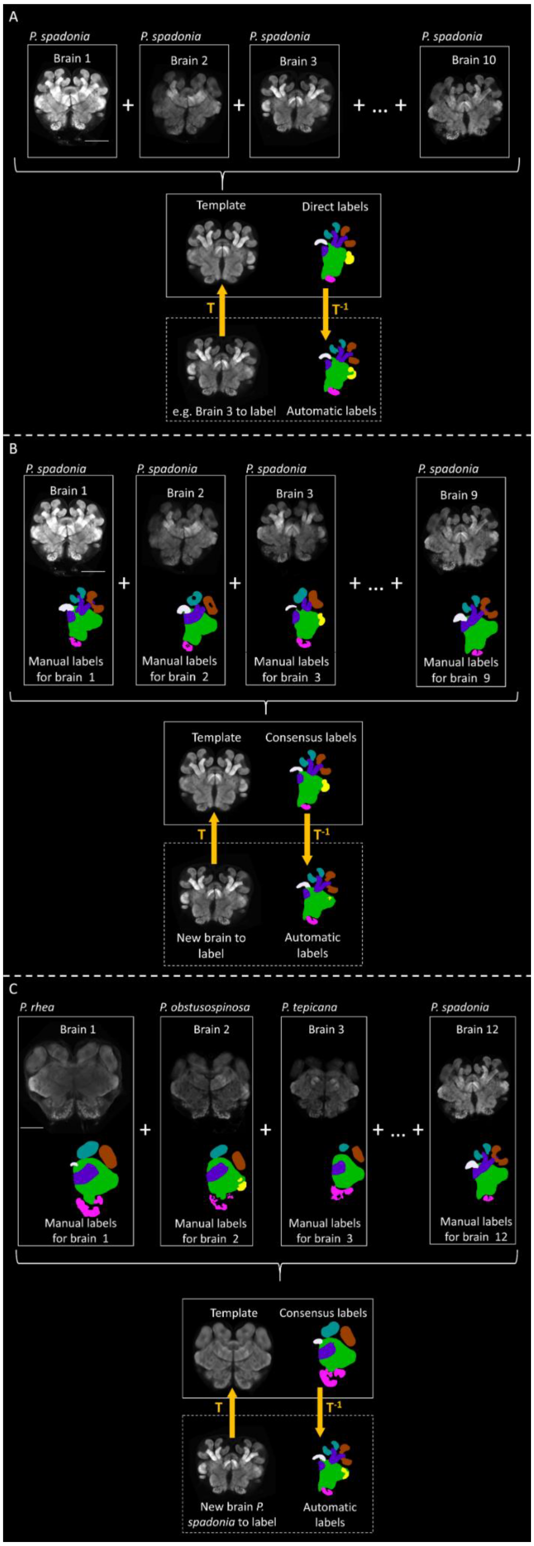

FIGURE 2 | Automatic labeling methods. **(A)** Ten confocal images of brains of *P. spadonia* minors are combined on a single group-wise template, which is manually traced (creating "direct labels"). Each brain used to build the template (and other new brains) can be registered against the template with a transformation function *T*. The inverse function T^{-1} can be used on the manual labels of the template to automatically label the registered brain. **(B)** Nine confocal images of brains of *P. spadonia* minors are combined on a single group-wise template. The existing manual labels of each brain are registered against the template, and every voxel is assigned to one label by majority voting (creating "consensus labels"). New brains can be registered against the template with a transformation function *T*. The inverse function T^{-1} can be used on the consensus labels of the template to automatically label the registered brain. **(C)** Twelve confocal images of brains of *P. spadonia*, *P. rhea*, *P. tepicana*, and *P. obtusospinosa* (three of each species) are combined on a single multispecies group-wise template. Consensus labels are created for the template as in B, and the same procedure is applied to automatically trace new brains. Scale bar = 100 um. A single slice per brain has been shown for illustration clarity.

on a two-step approach using symmetric diffeomorphic image registration[2] (SyN, Avants et al., 2008) of a group of gray value brain images to one another by maximizing mutual information[3] first and cross-correlation[4] later. Following this optimization process, the group of images are warped into the same coordinate system. In the first step, all gray value brain images are registered against one randomly selected image by optimizing mutual information and allowing only affine transformations (translations and proportional changes in size). Transformed images are then averaged to build a preliminary "blurry" reference brain image. In the second step, the original gray value brain images are registered to this blurry average using non-rigid transformations (i.e., allowing local deformations) by maximizing the cross-correlation of the intensities of all brains. In this step, the registration is gradually improved at four resolution levels (sequentially at ⅛, ¼, ½, and 1/1 of the original sizes, following a resolution pyramid strategy) and produces an optimal average template. The first registration compensates for large disparities in size while the second locally finds an optimal solution. The template was generated by the normalized voxel-wise median of the co-registered volumes (Arganda-Carreras et al., 2017). All steps were implemented in the Advanced Normalization Tools (ANTs) software (Avants et al., 2011) after transforming in Fiji (Schindelin et al., 2012) gray and label images to the open format NRRD. For a detailed description of the software methods used in this paper, we refer the reader to **Supplementary Material**.

Seven group-wise templates were generated for this study (**Supplementary Table 1**) with 9 ("consensus label method"), 10 ("direct label method") or 12 (multispecies template method) original gray value brain images. Six of them were single-species templates, built using only *P. spadonia* minor gray value brain images ("direct/consensus label methods"). One was a hybrid template, generated from brains of *P. spadonia*, *P. rhea*, *P. tepicana*, and *P. obtusospinosa* minors ("multispecies

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[2] **Image registration:** process of transforming one image (usually known as moving image) into the coordinate system of another image (usually known as fixed image).

[3] **Mutual information:** metric taken from information theory and used on image registration to measure the amount of information that one image contains about another image. It should be maximum when both image are perfectly aligned.

[4] **Cross-correlation**: metric of the similarity of two images as a function of the displacement of one with respect to the other.

template method"), three brains per species. Templates were also associated with anatomical brain label values obtained either by manual or consensus labeling (see below).

Neuropil Labeling

Manual Labeling of Original Brain Images and Template

For each original gray value brain, an expert annotator determined the region occupied by each brain compartment by labeling them manually using Amira (version 6.0 or 2019.2). Labels were traced on eight compartments (as in Muscedere and Traniello, 2012; Gordon et al., 2017): the optic lobes (OL, comprising lobula, medulla and lamina and connecting fibers), antennal lobes (AL, comprising glormeruli, and central hub), mushroom-body medial calyx (MB-MC), mushroom-body lateral calyx (MB-LC), mushroom-body peduncle (MB-P), central complex (CX, comprising the lower and upper division of the central body, the protocerebral bridge and the noduli), subesophageal zone (SEZ) and rest of the central brain (ROCB). This manual tracing was performed in only one brain hemisphere, except for the CX, SEZ and ROCB, which lack a clear subdivision between hemispheres. A trained annotator requires approximately 1 h to label a brain hemisphere. **Figure 1** shows three confocal scans of a *P. spadonia* brain. Studies aiming to analyze differences between the right and the left sides of the brains would require, however, to have fully traced brains.

A single dataset of manual labels for the template generated for the "direct label method" was obtained using the same methodology described above.

Consensus Labeling of Templates

One method used to obtain the same regional label values on the group-wise template is based on combining the information provided by the manual label values of the original brains used to build the template, which also needed to be transformed to the NRRD format. The first step consisted of applying to each label image the same diffeomorphic transformations performed on its original brain anatomy (T^1, **Figures 2B,C**), and later a per-voxel majority voting over all deformed label images of the same brain center to produce "consensus labels." Since not all the samples of our original dataset contained labels of the same hemisphere, we used Fiji's tool "Flip horizontally" (Schindelin et al., 2012) when needed to create mirror images of brain anatomies and their manual labels to only have samples with right-hemisphere labels.

Automatic Labeling of Original Brain Images

To automatically label gray value brain images, individual brain images were registered against a group-wise template performing the same two-step method described above—initial affine registration maximizing mutual information followed by a non-rigid registration optimizing cross-correlation. The inverse transformations (T^{-1}, **Figure 2**) were then applied to the template regional labels (regardless of the method chosen to generate them), automatically building label values for individual gray value brain images registered against the template. To avoid always tracing the same side and prevent bias due to natural brain asymmetries, a proportion of the gray value brain image datasets to be traced can be flipped.

Five *P. spadonia* gray value brain images were automatically traced using the three methods described before. It is important to notice that for the "direct label method," these five gray value brain images were also used to build the template, while for the other two methods, which use consensus labels, these five brains were left out of the templates. This is because the consensus labels integrate the information from the manual labels of the brain anatomies used for the template: on one hand, it would seem unnecessary to relabel those brains, and on the other hand, the original manual labels and the automatically obtained labels would be basically the same and the objectivity of the evaluation of the method would be compromised.

Evaluation of Approaches

Because automatic and manual labels are expected to produce slightly different results, we needed to determine whether these differences were acceptable. To do so, we compared differences between automatic and manual labels with the differences between manual labels generated by several expert annotators ("Inter-Person") and by the same annotator ("Intra-Person") tracing the same gray value brain image more than once (**Supplementary Table 3**). Three annotators (with at least 2 years of experience tracing brains) traced the same five brains (to have an acceptable measure of interpersonal differences, "Inter-Person"), and one of them traced the same five brains three times (to have an acceptable measure of intrapersonal differences, "Intra-Person"). The three expert annotators also traced the single species (*P. spadonia*) template for the "direct label method." As explained for consensus label creation, when manual labels were on the left side, the gray value brain anatomy and the labels were flipped to be on the right side.

Because many comparative neuroanatomical studies use volumetric data, as a measure of neuropil investment (Wehner et al., 2007; Muscedere and Traniello, 2012; Riveros et al., 2012; O'Donnell et al., 2018; Arganda et al., 2020), Volume similarity (Eq. 1) was used as the relevant metric for evaluating automatic labeling methods and was calculated for each label and brain, as well as for the total brain volume, using volumes estimated with the open-source toolbox MorphoLibJ (Legland et al., 2016; see **Supplementary Table 2**).

$$\textit{Volume similarity} = 2 \times \frac{\left|\textit{Volume label method } 1 - \textit{Volume label method } 2\right|}{\left|\textit{Volume label method } 1 + \textit{Volume label method } 2\right|} \quad (1)$$

Volume similarity between labels annotated for the same compartment obtained by different methods was calculated within the same gray value brain image, and for the automatic and manual labels pairing labels always related to the same original annotator (e.g., OL volume obtained by the "multispecies template" strategy using consensus labels built from manual labels by annotator 1 + OL volume obtained by manual labels from annotator 1; see **Supplementary Table 3**).

Statistical Analysis

We used bootstrapping to perform statistical analyses (Efron and Tibshirani, 1994). This method has the advantage of making no assumptions about the distributions underlying the data and of being able to handle datasets where data are not fully independent, as is the case in our dataset for different measurements performed on the same brain. To make pairwise comparisons between volume similarity measurements of one brain center provided by two methods, we first we selected one brain at random and pooled all volume similarity measurements for the same brain center from the control and the method. From this pool, we selected volume similarity measurements randomly and with replacement, creating two randomized sets of measurements, with the same sizes as the originals. We then selected a new brain at random with replacement (the same brain can be selected several times) and repeated the same procedure 5 times because our dataset to evaluate the methods has a total of 5 brains. We thus obtained a randomized dataset with the same statistical characteristics as the original, but in which measurements in the two groups came from the same distribution. We then computed the difference between the means of the measurements of the two groups, *d_rand*. We repeated this procedure 10,000 times, obtaining a distribution for *d_rand*. This distribution is centered at 0 by construction, and its width represents the differences between method and control that we could expect by chance if both belonged to the same distribution. We then computed the difference between each method and control from the dataset and defined our *p*-value as the proportion of *d_rand* that had a value greater than the actual difference between the two methods found in our study. We set the significance level at $p < 0.05$.

RESULTS

We compared the variability (measured as volume similarity) between automatic methods ("Direct labels," "Consensus labels," and "Multispecies template") and expert annotators, to the variability among ("Inter-Person") and within ("Intra-Person") annotators (**Figure 3** and **Supplementary Table 4**). This allows the determination of whether the differences between automatic and manual labels are comparable to those produced by expert annotators that we accept as inevitable errors. Regardless of the comparisons between automatic and manual methods, our results showed that the inter- and intra-individual differences can be considerable, reaching ca. 10% and even higher in compartments such as the AL and the CB (**Figure 3**).

In general, we found that, regardless of method, differences between automatic and manual labels were similar to inter- and intra-individual tracing variability, and in some cases actually smaller. This indicate that automatic methods were more reliable than having different annotators or the same annotator repeat the labels. Compared with inter-person variability for the same compartments, the variability of the "direct labels method" was 5% smaller in the OL (**Figure 3A**, *p*-value = 0.028, **Supplementary Table 4**), 11% smaller in the AL (**Figure 3B**, *p*-value = 0.025, **Supplementary Table 4**), and 5% smaller in the MB-P (**Figure 3E**, *p*-value = 0.01, **Supplementary Table 4**).

The variability of the "multispecies template method" was 4% larger than the inter-person variability only for the ROCB (**Figure 3H**, $p = 0.025$, **Supplementary Table 4**). When comparing the automatic methods with the intra-individual variability, larger variabilities of the automatic methods were found for the MB-MC, in which the variability of "direct label method" was 5% larger (**Figure 3C**, $p = 0.026$, **Supplementary Table 4**), for the ROCB, in which the variability of the "direct label method" was 2% larger (**Figure 3E**, *p*-value = 0.004, **Supplementary Table 4**) and the variability of the "multispecies template method" was 6% larger (**Figure 3E**, *p*-value = 0.015, **Supplementary Table 4**). A marginally significant difference (8% smaller, **Supplementary Table 4**) was found when comparing the "consensus label method" and the intra-person variabilities.

The "consensus label method" produced variabilities similar to those among and within annotators for all compartments. Some differences were marginally significant (**Supplementary Table 4**) in comparison to the variability among annotators (5% smaller in the OL, 4% larger in the MB-LC, and 6% smaller in the CX) and within the same annotator (4% larger in the MB-LC and 3% larger in the SEZ, the ROCB and for the whole brain).

DISCUSSION

Statistical templates serve as representative neuroanatomies that integrate variation in brain structure across samples. When associated with neuroanatomical labels, they are a valuable tool to automatically and efficiently segment compartments in similar brains that have not been previously traced. With these annotations we can calculate descriptive metrics such as brain compartment volumes useful to understand differential investment in brain centers and their associated neural functions in behavior.

We presented and evaluated three methods to determine whether their results are comparable to manual annotations. To do so, we compared volumetric differences between automatic and manual labels to volumetric differences due to inter- and intra-individual variability of annotators. We found that automatic segmentation produced satisfactory results. Our three automatic methods produced compartmental volumetric data similar to those obtained via manual annotations by different annotators or by the same annotator repeatedly tracing the same brain. In some cases, we found that the variability between automatic and manual data was even smaller than inter-person variability. Only for one center evaluated (ROCB), the "multispecies template method" produced a variability 2% larger than the inter-person one. This error level might be acceptable considering the benefits of automation and the reduction in human bias. We expected to find more differences in comparison with intra-person variability. Surprisingly, only for two neuropils (MB-MC and ROCB), the "direct label method" and the "multispecies template method" produced larger differences (2–6% larger) between automatic and manual data than intra-person variability.

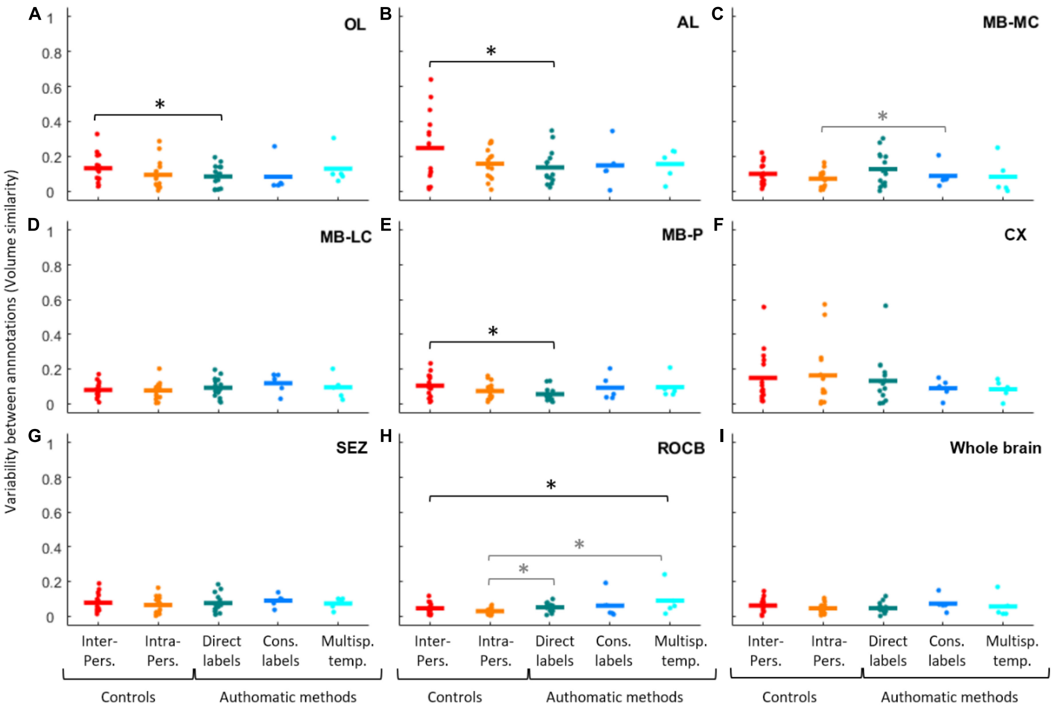

FIGURE 3 | Variability between annotations for brain compartments and the whole brain. Variation (using volume similarity) given is for the **(A)** optic lobe (OL). **(B)** Antennal lobe (AL). **(C)** Mushroom body medial calyx (MB-MC). **(D)** Mushroom body lateral calyx (MB-LC). **(E)** Mushroom peduncle (MB-P). **(F)** Central complex (CX). **(G)** Subesophageal zone (SEZ). **(H)** For the rest of the central brain (ROCB). **(I)** For the whole brain. Statistical comparisons are made using bootstrapping tests for comparing the volume differences found between the manual and the automatic labels ("Direct labels," "Cons. Labels," and "Multisp. temp.") and between individuals ("Inter-Person") or within the same individual ("Intra-Person"). "*" indicates p-values smaller than 0.05.

Standardized average brain atlases (group-wise templates) are increasingly applied in insects (Rein et al., 2002; Brandt et al., 2005; El Jundi et al., 2009; Kvello et al., 2009; Rybak et al., 2010; Peng et al., 2011; Menzel, 2012; Rybak, 2012; Costa et al., 2016; Arganda-Carreras et al., 2017, 2018; Gordon et al., 2019; Groothuis et al., 2019; el Jundi and Heinze, 2020) to efficiently and accurately collect data required to test hypotheses of brain evolution and to facilitate the establishment of connectomes. They allow, for example, the registration of multiple marked neurons into standard anatomies to determine their spatial relationships and possible inclusion in common neuronal circuits (e.g., Brandt et al., 2005; Peng et al., 2011). Annotated atlases also provide information on the shape and size of the different brain compartments to make intra and interspecific comparisons (e.g., Rein et al., 2002; Heinze et al., 2013; De Vries et al., 2017) and generate and test hypotheses on the importance of particular modalities of sensory processing in insect behavior, ecology, and sociobiology, and life history.

In ants, most brain studies present 3D models based on representative individuals (e.g., Bressan et al., 2015; Habenstein et al., 2020) instead of standardized brain atlases. Aside from accounting for interindividual variability and reducing the possible bias of a single representative, the use of group-wise templates allows the rapid and accurate collection of volumetric neuroanatomical data. To our knowledge, we were the first to generate group-wise templates and consensus labels in ants to automatically trace similar brains (Arganda-Carreras et al., 2017). In this work, we also presented for the first time a multispecies template. In another study, we used group-wise templates manually traced to reduce the time needed to trace 60 brains of three different brain phenotypes of the polymorphic turtle ant *Cephalotes varians* (Gordon et al., 2019). Here we validate these different methods using the variability of human annotations as the "gold standard." All the methods presented reduce the time required for manually tracing each brain and help decrease potential errors of multiple annotators, either by allocating a single annotator to a large dataset or by combining labels that integrate variability between samples. Group-wise templates also advantageously ensure blind annotations for samples of different origins known to the annotator (for example, different treatments or species) thus minimizing biases. For this purpose, we plan to build single templates for polymorphic species in future studies. Each strategy might be more suitable to answer some research questions than others; for example, the "direct label method" is recommendable for blind studies comparing individuals under different treatments. The "consensus label method" might provide with robust reference anatomical atlases that consider interindividual variability. And the "multispecies template method" can make evolutionary and comparative studies requiring large datasets from multiple species more robust. While our methods have been evaluated using descriptions of major neuropils, testing them on finer neuropil sub-structures will be a logical next step that will increase their potentiality. Regardless of the neuroanatomical scale, the use of templates to accurately and rapidly collect volumetric neuroanatomical data, combined with sociobiological, socioecological, phylogenetic, metabolic, or neurochemical analyses can help elucidate macroevolutionary and microevolutionary patterns of brain evolution. This will allow to better understand encephalization and allometric scaling in regard to the behavioral ecology and sociobiology of individual workers, and the impact of emergent colony-level processes on the brain.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

DG prepared the brains for imaging, registered images, and manually labeled all brain images and the templates. AH manually labeled a sample of brain images and the templates. SA manually labeled a sample of brain images and the templates and prepared all brain images for template creation. IA-C implemented the software to create the templates and to automatically label brain subregions. SA and AP-E designed the methodology to evaluate automatic labeling and performed the statistical analysis. SA, IA-C, and JT conceptualized and designed the study and wrote the first draft of the manuscript. SA, IA-C, MG, and JT and secured funding. All authors edited and approved the final content of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.745707/full#supplementary-material

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Social Cues of Safety Can Override Differences in Threat Level

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Animals in groups integrate social with directly gathered information about the environment to guide decisions regarding reproduction, foraging, and defence against predatory threats. In the context of predation, usage of social information has acute fitness benefits, aiding the detection of predators, the mounting of concerted defensive responses, or allowing the inference of safety, permitting other beneficial behaviors, such as foraging for food. We previously showed that *Drosophila melanogaster* exposed to an inescapable visual threat use freezing by surrounding flies as a cue of danger and movement resumption as a cue of safety. Moreover, group responses were primarily guided by the safety cues, resulting in a net social buffering effect, i.e., a graded decrease in freezing behavior with increasing group sizes, similar to other animals. Whether and how different threat levels affect the use of social cues to guide defense responses remains elusive. Here, we investigated this issue by exposing flies individually and in groups to two threat imminences using looms of different speeds. We showed that freezing responses are stronger to the faster looms regardless of social condition. However, social buffering was stronger for groups exposed to the fast looms, such that the increase in freezing caused by the higher threat was less prominent in flies tested in groups than those tested individually. Through artificial control of movement, we created groups composed of moving and freezing flies and by varying group composition, we titrated the motion cues that surrounding flies produce, which were held constant across threat levels. We found that the same level of safety motion cues had a bigger weight on the flies' decisions when these were exposed to the higher threat, thus overriding differences in perceived threat levels. These findings shed light on the "safety in numbers" effect, revealing the modulation of the saliency of social safety cues across threat intensities, a possible mechanism to regulate costly defensive responses.

Keywords: defensive behavior, freezing, social buffering, *Drosophila melanogaster*, motion cues, safety in numbers, fear, looming stimulus

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INTRODUCTION

A major benefit of being in a group is the possibility of adding social information to directly perceived information about the environment to guide behavior. Across the animal kingdom, this social information can not only be actively transmitted *via* signals evolved specifically for communication (Hollén and Radford, 2009; Leonhardt et al., 2016), but also acquired through information-bearing cues of different sensory natures, which animals produce as they engage in their daily activities. Vertebrates use such social cues to procure food (Galef and Giraldeau, 2001), for example, using vision to assess where and how much others are eating (Coolen et al., 2005), to choose mates by copying the decisions of others (Kavaliers et al., 2017) based, for example, on olfactory cues (Galef and Laland, 2005), and to infer predation threat levels (Griffin, 2004), for instance, by auditory detection of escape (Murray et al., 2017) or freezing (active immobility response aimed at becoming inconspicuous) (Pereira et al., 2012). These types of social cue usage are also reported in invertebrates, including *Drosophila melanogaster* (Ferreira and Moita, 2019; Couzin-Fuchs and Ayali, 2021), guiding aggregation on food (Tinette et al., 2004; Dombrovski et al., 2017, 2019; Shultzaberger et al., 2018), reproduction-related decisions in mating (Mery et al., 2009; Danchin et al., 2018) and oviposition (Sarin and Dukas, 2009; Battesti et al., 2012; Bailly et al., 2021), as well as defensive responses (Ferreira and Moita, 2020), many of which rely at least partially on vision.

The acquisition and exploitation of the information provided by social cues can confer fitness benefits (Kendal et al., 2005), particularly in the context of a response to a potential threat: failure to detect a predator can lead to an animal's immediate demise, whereas needless engagement in metabolically costly defense responses (Barrios et al., 2021) can negatively impact survival. That individual defense responses to predatory threats can vary in modality and vigor depending on the perceived threat level is well known (De Franceschi et al., 2016). Surprisingly, however, detailed studies of how different threat levels impact group behavior are still scarce. There are reports of modulation of group responses with threat, showing that prey species from higher-predation habitats form larger and more cohesive groups than those from lower predation environments (Seghers, 1974; Hager and Helfman, 1991; Magurran et al., 1992; Beauchamp, 2004). At the interplay of foraging and predation, there are further examples of modulation by threat level of reliance on social cues (Kendal et al., 2005). For example, bumblebees use the presence of conspecifics as a cue of safety, joining others at foraging sites only in potentially hazardous situations, when those sites were previously predator-infested (Dawson and Chittka, 2014). Minnows also use socially derived information and copy feeding location to a higher extent when exposed to a higher predation risk (Webster and Laland, 2008), an example of the "copy-when-asocial-learning-is-costly" hypothesis regarding social learning. These examples point to the fact that group foraging behaviors are modulated by threat level, and that reliance on social cues also varies with predation risk. Although it has been reported that the angle of approach of a threat can trigger different degrees of uniformity in escape formations (Marras et al., 2011), little is known regarding how different threat levels affect group defensive responses and reliance on social cues, which we addressed here.

To study responses to threat, we used visual looming stimuli mimicking an approaching predator, which have been reported to elicit defensive behaviors in all visual animals tested so far, from invertebrates, such as crabs (Oliva et al., 2007) and flies (Card and Dickinson, 2008; Zacarias et al., 2018), to vertebrates like mice (Yilmaz and Meister, 2013) and humans (Ball and Tronick, 1970). Using visual threats, whose properties can easily be manipulated in the lab, permits a detailed understanding of how different aspects of a threat affect the crucial deployment of defense responses. For example, a black disk sweeping overhead, mimicking a cruising predator, elicits freezing, while the same black disk expanding on the screen, as if looming toward the mouse, induces escapes (De Franceschi et al., 2016). Similarly, fruit flies have been shown to respond both with escapes and with freezing to repeated, inescapable, sweeping, and looming stimuli, where escapes predominate in response to sweeps (Gibson et al., 2015) and freezing in response to looms (Zacarias et al., 2018). In addition, in both zebrafish larvae (Bhattacharyya et al., 2017) and flies (Card, 2012; von Reyn et al., 2017), looming stimuli with lower approach rates evoke slower escapes, while higher approach rates evoke faster responses.

We had previously shown that flies exposed to looming stimuli in groups use social motion cues as both a cue of threat and safety (Ferreira and Moita, 2020). On the one hand, freezing in others leads to freezing in a focal fly. On the other hand, in line with buffering effects in other animals (Kiyokawa et al., 2004, 2014; Faustino et al., 2017), movement of others leads to movement resumption after freezing. In this study, we addressed how different threat levels affect group freezing behavior and usage of social motion cues. We used two loom speeds as different degrees of threat and analyzed freezing responses in individual and group tested flies to uncover the effect of threat imminence on group-mediated freezing responses. We then manipulated the social environment, controlling the numbers of moving and freezing flies surrounding a focal fly to clearly disentangle the effect of looming speed on the usage of social motion cues in regulating freezing responses.

MATERIALS AND METHODS

Fly Lines and Husbandry

Flies were kept at 25°C and 70% humidity in a 12:12 h dark:light cycle. Experimental animals were mated females, tested only once when 4–6 days old. For optogenetic manipulations, flies were transferred for 48 h before the experiments to food with 0.4 mmol/L retinal, a required co-factor for the function of the opsin CsChrimson. In experiments with mixed genotypes, focal flies were marked on the thorax using a white marker pen.

Wild-type flies used were Canton-S. *LC6-splitGAL4* line *w[1118]; P{y[+ t7.7] w[+ mC] = R92B02-p65.AD}attP40; P{y[+ t7.7] w[+ mC] = R41C07-GAL4.DBD}attP2* (Wu et al., 2016) and *w[*] norpA[36]* (blind flies) were obtained from the Bloomington stock center. The *UAS-CsChrimson* line used was w^{1118}; *P{20XUAS-IVS-CsChrimson.mVenus}attP2* (Klapoetke et al., 2014). *LC6-splitGal4* driver-line flies were crossed with *UAS-CsChrimson* effector flies, to create LC6 > CsChrimson flies used for optogenetic induction of freezing (freezing flies).

Behavioral Apparatus and Experimental Protocol

Behavioral experiments were performed as described in (Ferreira and Moita, 2020), with minor modification. Briefly, we imaged unrestrained flies in 11° slanted polyethylene terephthalate (PETG) arenas with 68 mm diameter (central flat portion diameter 32 mm). A screen (Asus monitor running at 240 Hz, tilted 45° over the stage, **Figure 1A**) was used as a source of light and the delivery of looming stimuli. The experiments ran for 10 mins comprising a 5-min baseline where the screen was kept white, followed by a 5-min stimulation period where twenty 500 ms looming stimuli were delivered at random intervals ranging from 10 to 20 s [black circles in a white background, with a virtual object length of 1 cm (Zacarias et al., 2018), exponentially expanding at a speed of 25 or 50 cm/s; half length to speed ratios, l/v, values of 40 and 20 ms, respectively, corresponding to biologically relevant threats, which mimic damselfly attacks (von Reyn et al., 2014)]. The stage contained two arenas, and under it, a custom-built LED board that provided invisible backlight for video imaging (infrared, 940 nm) and

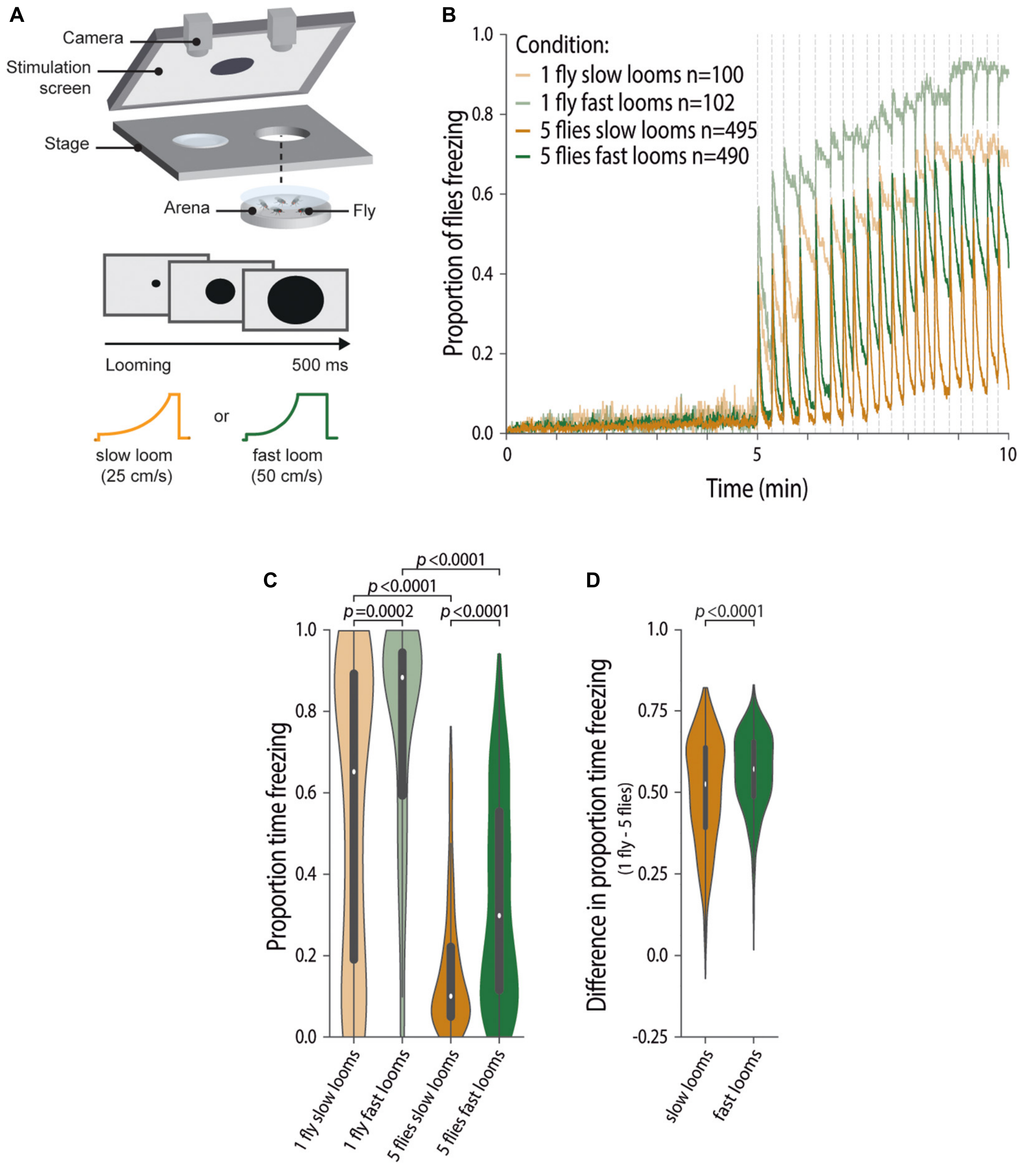

FIGURE 1 | Group freezing responses scale with threat imminence. **(A)** Experimental setup and conditions: we tested individuals and groups of five flies in backlit arenas imaged from above; after a 5-min baseline, flies were exposed to twenty 500 ms looming presentations, every 10–20 s; we provided either slow (25 cm/s, purple) or fast looms (50 cm/s, green). **(B,C)** Data for flies tested individually (lighter shades) and in groups (darker shades). **(B)** Fraction of flies freezing throughout the experiment; dashed lines represent looming stimuli presentations; *n* represents the numbers of flies tested for each condition. **(C,D)** Violin plots representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: [central white dot, median; box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. **(C)** Proportion of time spent freezing in the stimulation period. *P*-values result from Kruskal–Wallis statistical analysis followed by Dunn's multiple comparisons test. **(D)** Difference in the proportion of time spent freezing between individually tested flies and flies tested in groups for slow and fast looms (refer to the section "Materials and Methods"). *P*-value results from the two-tailed Mann–Whitney test.

red-light (627 nm) for optogenetic stimulation. Videos were acquired through Bonsai (Lopes et al., 2015) at 60 Hz and 1,280 width × 960 height resolution using two USB3 cameras (PointGrey Flea3). We tested wild-type flies alone and in groups of five, as well as one focal wild type (marked with white paint) surrounded by different proportions of blind, moving flies, and optogenetically manipulated, freezing flies.

Optogenetic stimulation followed two protocols and all LC6 > CsChrimson flies within the same protocol received the same stimulation: (1) stimulation without concurrent loom presentations–after a 5-min baseline period, 20 stimuli of pulsed red light at 50 Hz, 50% duty cycle (DC), 7.5 mw/cm^2 normalized intensity were delivered over the course of another 5 min and (2) optogenetic manipulations with simultaneous looming stimuli–again after a 5-min baseline period, coinciding with the initiation of the presentation of the looms stimulation occurred over 2 min, at 50 Hz, 50% DC, 10 mW/cm^2 normalized intensity.

Data Analysis

Data were analyzed using custom scripts in spyder (python 3.8). Statistical testing was done in GraphPad Prism 7.03, and non-parametric, Kruskal–Wallis test followed by Dunn's multiple comparison test or two-tailed Mann–Whitney test were chosen, as data were not normally distributed (Shapiro–Wilk test).

As reported in our previous study (Ferreira and Moita, 2020), we used IdTracker (Pérez-Escudero et al., 2014) to obtain the position of each individual fly throughout the video, and hence acquire x, y coordinates, and then used a costume Bonsai (Lopes et al., 2015) script to analyze motion and hence pixel change a 4 mm × 4 mm square around the center of mass of the fly. With these metrics, as described previously (Zacarias et al., 2018; Ferreira and Moita, 2020), we were able to classify different behaviors, taking into account pixel change and speed, namely, freezing and jumps.

Freezing bouts were classified as zero-pixel change detected around the fly for at least 500 ms (30 frames). Noise in the images can create pixel changes even when the fly is still visibly immobile. Therefore, to decrease the incidence of false freezing breaks (where the fly is still freezing but noise in the image creates pixel changes), within a bout of freezing, pixel changes occurring for less than 50 ms (3 frames) were allowed, that is only pixel changes detected for more than this period were considered true breaks in freezing.

Freezing in response to looms was determined using a time window starting 30 frames before each loom until 150 frames after the loom. The probability of freezing entries (or freezing onset) was calculated by determining the likelihood a fly that was not freezing 30 frames before a loom, started freezing during, or until 150 frames after it. Latency to freeze corresponds to the time from loom onset to the initiation of freezing (depicted in **Supplementary Figures 1A,B**). The probability of freezing exits (or freezing offset) between looms was calculated by determining the fraction of instances that flies were freezing within the 2 s after the loom and were not freezing in the last 0.5 s before the next loom (meaning they broke freezing in between looms).

The proportion of time spent freezing was quantified by taking the sum of the frames in which freezing occurred during the stimulation period (5 min, corresponding × frames) and dividing that by the total number of frames of this period. Flies typically freeze in bouts, initiating freezing around the end of the looming stimulus and remaining immobile for different lengths of time. The length of each freezing bout varies across looming stimuli and across flies, such that the same proportion of time freezing may result from different freezing patterns. Therefore, we also analyzed the distribution of freezing bout lengths, by measuring the time elapsed from freezing onset to offset or until the experiment ended (when flies once freezing remain immobile for the rest of the test session).

To determine when a jump occurred, we identified when a fly's speed exceeded 75 mm/s for at least one frame, and applied a time constraint of 3 frames between two consecutive jumps (Zacarias et al., 2018; Ferreira and Moita, 2020).

To compare the effect size of a manipulation across conditions, for example, to compare the effect of manipulating social environment (individually vs. group tested flies) across threat level (slow vs. fast looms), we used the following strategy, as the use of common effect size statistics in non-parametric data is controversial: first, we took the median value of the proportion of time spent freezing by flies tested individually (from a sample of 20 flies exposed to slow looms) and subtracted the median value of time spent freezing by flies tested in groups (again a sample of 20 flies exposed to slow looms was used). We repeated this procedure 1,000 times (each time a random sample, with replacement, of 20 individuals was used), creating a distribution of difference values in the proportion of time spent freezing between flies tested individually and in groups, when exposed to slow looms. In this manner, we simulated 1,000 replicates of this experiment, allowing for an estimation of the effect size of manipulating the social environment of flies exposed to one threat level, i.e., slow looms. Next, we performed the same for flies exposed to fast looms, and a distribution of the difference between social conditions in the proportion of time spent freezing by flies tested under a higher threat level was generated. Finally, we compared the distributions thus generated, allowing for a comparison of the effect size of manipulating social condition across loom speeds. A similar procedure was performed to compare the effect size of manipulating threat level across social conditions. The same approach was used when analyzing freezing exits.

As previously described (Ferreira and Moita, 2020), we calculated motion cues for a focal as the summed product of speed and angle on the retina of a focal fly that each of the surrounding flies produces $\sum speed = angle\ on\ the\ retina$ (θ) where $\theta = 2\,arctan\left(\frac{size}{2 \times distance}\right)$.

Logistic Regression Model

We modeled the decision to stay frozen or resume movement using the scikit-learn logistic regression model, as previously described (Ferreira and Moita, 2020). Briefly, we modeled the probability of exiting freezing in between looming stimuli as a function of the looming speed and the average of the sum of the motion cue generated by neighboring flies during that freezing bout. We used 100,000 times bootstrapped data with replacement. To determine the explanatory power of each predictor, we determined the associated fraction of variance.

RESULTS

To study how different threat imminences affect social defensive responses of the flies, we first assessed whether the speed with which a looming dark disk approaches modulates freezing responses of individual flies. To this end, we compared freezing behavior of individually tested flies exposed to one of two looming speeds, 25 and 50 cm/s (**Figure 1A**). A pilot experiment suggested that individual freezing responses varied with looming speed and, indeed, we found that the fraction of flies freezing throughout the experiment when exposed to twenty renditions of the slower loom (25 cm/s) is inferior to that observed when flies are exposed to the same number of renditions at a faster speed (50 cm/s) (**Figure 1B**). This is further corroborated by the increased time spent freezing by flies exposed to the faster looms compared with flies exposed to the slower looms (Proportion of flies Freezing, $\text{PropF}_{25} = 0.65$ IQR 0.18–0.89, $\text{PropF}_{50} = 0.88$ IQR 0.58–0.94, Kruskal–Wallis, KW, followed by Dunn's multiple comparisons test, D, $p = 0.0002$, $Z = 4.12$; **Figure 1C**). Exposure to faster looms also led to more rapid freezing responses, as revealed by the shorter latencies to freeze in response to the fast looms relative to slow looms (**Supplementary Figure 1A**). Having established that increasing looming speed increases freezing responses in flies tested individually, we will henceforth use looming speed to study how different levels of threat affect defensive behaviors in groups.

As flies show social buffering of defensive responses, that is, when exposed to a threat while surrounded by others they freeze less (Ferreira and Moita, 2020) and groups of animals can behave differently depending on the level of threat (Seghers, 1974; Hager and Helfman, 1991; Magurran et al., 1992; Beauchamp, 2004; Marras et al., 2011), we hypothesized that experiencing looms of different speeds impacts group behavior and the weights given to the available social information. We thus compared freezing responses of flies, tested in groups of five, exposed to the fast and slow looms (**Figures 1B,C**) and found that, just as individually tested flies, groups of five flies exposed to the faster looms freeze more than groups exposed to slower looms ($\text{PropF}_{25} = 0.10$ IQR 0.50–0.22, $\text{PropF}_{50} = 0.30$ IQR 0.11–0.55, KWD $p < 0.0001$, $Z = 10.35$; **Figure 1C**). In addition, flies exposed to fast looms in groups show shorter latencies to start freezing that are similar to those observed for flies tested individually (**Supplementary Figure 1B**). Although both flies tested individually and flies tested in groups responded with more sustained freezing to the faster loom, it is still possible that the impact of the social environment, that is, the degree of social buffering, varied with threat level. To test this possibility, we compared the decrease in freezing of flies tested in groups relative to freezing levels of individually tested flies for both loom speeds (refer to the section "Materials and Methods," **Figure 1D**). We found that the decrease in freezing, caused by social buffering, was slightly but reliably bigger for flies exposed to fast looms (two-tailed Mann-Whitney, MW, $p < 0.0001$). In summary, the time flies spend freezing scales with perceived threat imminence, but the social environment seems to have a higher weight, albeit to a small extent, in guiding freezing responses when flies are exposed to faster looms.

When in a group, the movement generated by the neighboring flies leads to an increase in freezing exits resulting in faster resumption of activity and less sustained freezing in between loom presentations (Ferreira and Moita, 2020). Hence, we analyzed the effect of looming speed on the probability of exiting freezing (**Figure 2**). Consistent with our previous results, flies tested individually display low probability of exiting freezing [$\text{Prob(Fexit)}_{25} = 0.21$, IQR 0.06–0.67, $\text{Prob(Fexit)}_{50} = 0.08$ IQR 0.05–0.17; **Figure 2A**], and flies tested in groups are more likely to stop freezing in between looming stimuli [$\text{Prob(Fexit)}_{25} = 0.94$ IQR 0.82–1.00, KWD $p < 0.0001$, $Z = 11.78$, $\text{Prob(Fexit)}_{50} = 0.79$ IQR 0.44–0.94, KWD $p < 0.0001$, $Z = 11.55$; **Figure 2A**]. In line with the results for the proportion of time spent freezing (**Figure 1**), perceived threat imminence significantly affects the probability of exiting freezing in flies tested individually (KWD $p = 0.0004$; **Figure 2A**) and in groups (KWD $p < 0.0001$, $Z = 3.92$; **Figure 2A**).

To further explore the impact of threat level on the decision to stop freezing, we used a logistic regression model. In our prior study (Ferreira and Moita, 2020) such a model revealed that the motion cue generated by surrounding flies was the strongest predictor of the decision to stop freezing, explaining close to 90% of the variance in the data. Therefore, in this study, we modeled freezing exits using the motion cue of others and looming speed as predictors (refer to the section "Materials and Methods," **Figure 2B**). This model accurately describes our data, as seen by the area under the receiver operating characteristic (AUROC), a measurement of model accuracy (0.72 ± 0.011), and shows that although the social motion cue explains most of the variance in the data (0.66 ± 0.041), looming speed also explains a significant part (0.33 ± 0.041). This model does not, however, allow us to look at the interaction between looming speed and the impact of social environment on the decision to stop freezing and resume activity. To address this issue, we compared the impact of the social environment on freezing exits observed for flies exposed to fast and slow looms, that is, we computed the difference P(Fexit)individual–P(Fexit)social, for flies exposed to both loom speeds, where a negative value means that there are more freezing exits in flies tested in groups. We found a small but reliable difference across loom speeds, as seen by the less negative values for flies exposed to the faster rather than to the slower looms (refer to the section "Materials and Methods," MW $p < 0.0001$, **Figure 2C**). Thus, the social environment had a stronger impact on freezing exits of flies exposed to slow looms, albeit to a small degree. As in our experimental conditions (in this and our prior study (Ferreira and Moita, 2020)) being surrounding by others leads to a buffering of freezing, it might be no surprise that this buffering effect is stronger under lower threat levels, which is in line with the finding that both for flies tested individually and for flies in groups, freezing bouts are shorter when exposed to slow looms than when exposed to fast looms (**Supplementary Figures 1C,D**). However, it stands in contrast with the stronger social impact

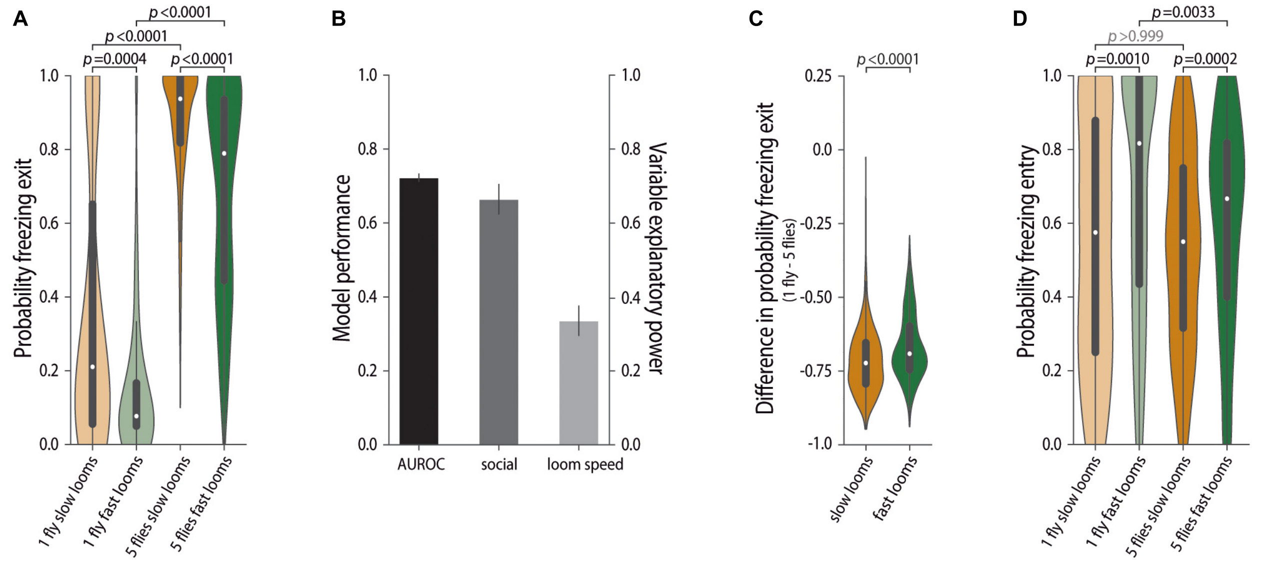

FIGURE 2 | Probability of freezing exit in groups scales with threat imminence. **(A,C,D)** Violin plots representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: central white dot, median; [box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. **(A)** Probability of freezing exit before the following looming stimulus. *P*-values result from Kruskal–Wallis statistical analysis followed by Dunn's multiple comparisons test. **(B)** Logistic regression model of the decision to stop or continue freezing as a function of social motion cues and looming speed (10,000 bootstrapping events). Mean and standard deviation of model performance (AUROC–area under the receiver operating characteristic curve, black) and explanatory power of the social environment (dark gray) and looming speed (light gray). **(C)** Difference in the probability of freezing exit between individually tested flies and flies tested in groups for slow and fast looms (refer to the section "Materials and Methods"). *P*-value results from the two-tailed Mann–Whitney test. **(D)** Probability of freezing entry upon looming stimulus. *P*-values result from Kruskal–Wallis statistical analysis followed by Dunn's multiple comparisons test.

on total time spent freezing for flies exposed to the fast loom. As the proportion of time spent freezing depends on both the probability of entering freezing upon a loom and the probability of breaking from freezing before the next loom, it is possible that while the social impact on freezing exits is bigger when flies are exposed to slow looms, the probability of entering freezing upon a loom may be decreased in groups to a larger extent during exposure to faster looms. Indeed, we found that flies exposed to slow looms individually or in groups are equally likely to enter freezing, whereas for flies exposed to fast looms, being in a group decreases the probability of freezing entry (KWD $p < 0.0033$, $Z = 3.456$, **Figure 2D**). This finding could result from a shift in balance of the weights given to social danger and safety cues as a function of threat level. In conclusion, looming speed affects both freezing entries and exits, an effect that may interact, even if weakly, with the impact of the social environment on freezing behavior.

Our results so far establish that both threat imminence and the social environment affect freezing responses. However, in these experiments, the social environment, i.e., the behavior of flies in the group, varies with threat level, as reflected in the increased average motion cue generated by groups of flies exposed to the slow looms relative to the motion cues produced by groups exposed to fast looms (**Supplementary Figure 2**). Therefore, to test the impact of threat imminence on the use of social cues, it is crucial to have experimental control over the social cues, namely, the motion of others, such that for different threat levels, the motion cues surrounding a focal test fly remain similar. To manipulate the social environment, we controlled the proportion of moving and freezing flies around a focal fly, from all four flies moving to all freezing and the various proportions in between. The same group compositions were exposed to the fast and slow looms (**Figure 3**). We used blind, NorpA mutant flies, which do not perceive the looming stimulus and walk the entirety of the experimental time, as the moving neighboring flies. This produces the highest surrounding motion cues, a manipulation we had previously shown to lower freezing by focal flies (Ferreira and Moita, 2020). To create freezing flies, we artificially induced freezing by optogenetically activating lobula columnar neurons 6 (LC6) using the channelrhodopsin CsChrimson (Klapoetke et al., 2014), LC6 > CsChrimson (**Supplementary Figure 3A**). Making use of these freezing and moving fly lines to create different proportions of moving and freezing flies (**Supplementary Figures 3B,C**), we were able to produce graded motion cues in groups for both looming speeds (**Figures 3A,B**). Crucially, these motion cues were similar across threat imminences (**Figure 3C**). However, optogenetically activating LC6 neurons, in addition to driving freezing, also triggers jumps coupled to the loom presentations, especially during the first 2 s of stimulation (**Supplementary Figures 3D,E**). This means that stable graded motion cues were present about 2 s after the first loom, being briefly interrupted upon loom onset subsequently (**Figures 3A,B**). It was therefore not possible with this manipulation to examine freezing as a social cue of threat, which mostly modulates freezing onset (Ferreira and Moita, 2020). However, the stable motion cues in between looming

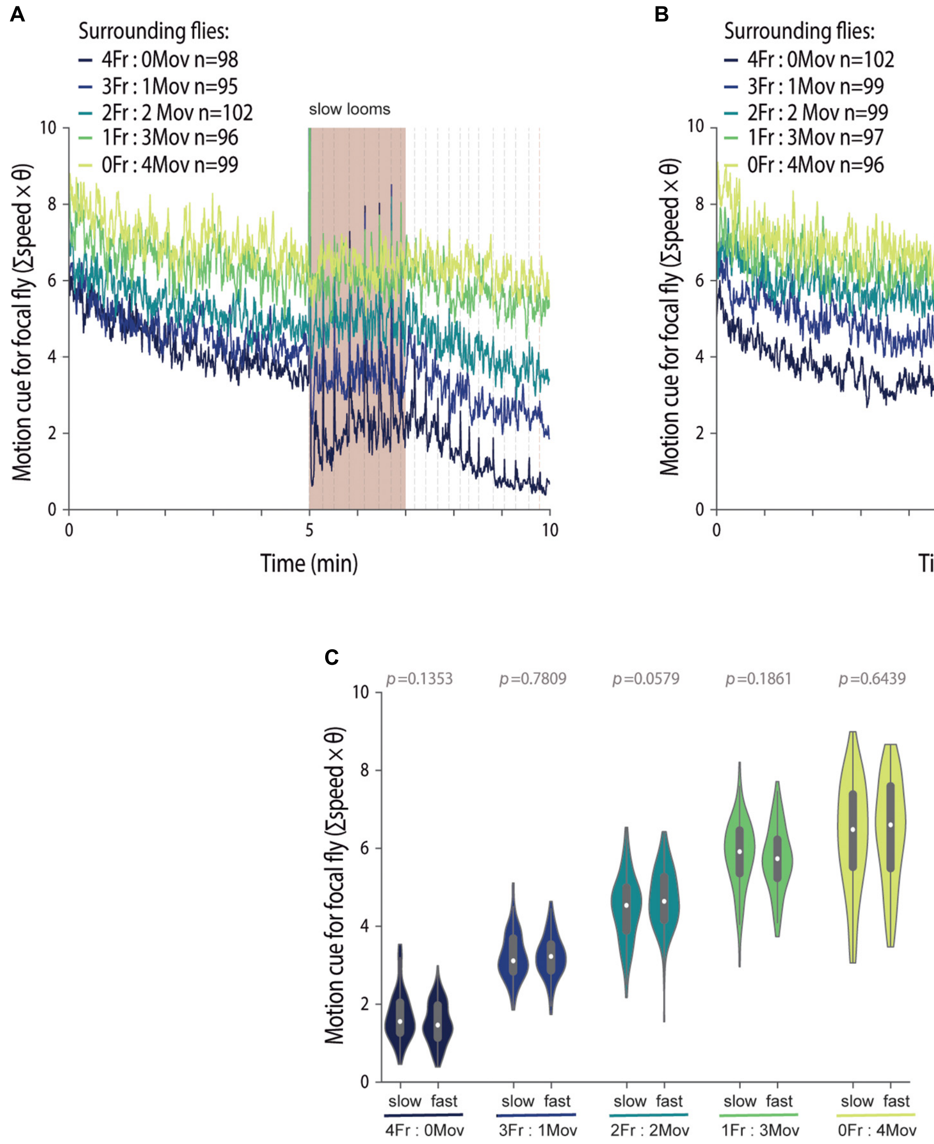

FIGURE 3 | Manipulating the social environment produces similar motion cues across threat imminences. **(A–C)** We manipulated four out of the five flies in a group, to surround focal flies with groups with different proportions of flies that always move (blind flies, NorpA) and flies that are optogenetically made to freeze (LC6 > CsChrimson). The color code for the groups is presented in **(A,B)**. Motion cues (refer to the section "Materials and Methods") produced by the manipulated surrounding flies throughout the experiment when exposed to slow **(A)** or fast looming stimuli **(B)**; dashed lines represent looming stimuli presentations; *n* represents the numbers of groups tested for each condition. **(C)** Violin plot representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: [central white dot, median; box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. Average motion cues produced by the manipulated surrounding flies during the stimulation period. *P*-values result from the two-tailed Mann–Whitney test; significance is determined *via* Bonferroni correction.

stimuli allowed the study of the use of safety cues, which modulate the resumption of activity by a fly that froze after the loom (Ferreira and Moita, 2020).

Having a handle on the social environment allowed us to analyze the behavior of focal wild-type flies exposed to similar social motion cues while being presented with looming stimuli of different speeds (**Figure 4**) and assess how different threat levels affect social cue usage. In both cases, we found that a graded manipulation of the social motion cues leads to graded freezing responses of focal flies, as seen in the proportion of flies freezing throughout the experiment (**Figures 4A,B**) and in the proportion of time each fly spends freezing (KW $p < 0.0001$, statistic = 127.5, **Figure 4C**; KW $p < 0.0001$, statistic = 196.8, **Figure 4D**). In addition, overall, flies exposed to faster looms freeze more than flies exposed to slower looms (**Figure 4C**). In opposition to our previous findings (Ferreira and Moita, 2020), flies surrounded by all freezing flies froze less than flies alone; we believe this is due to

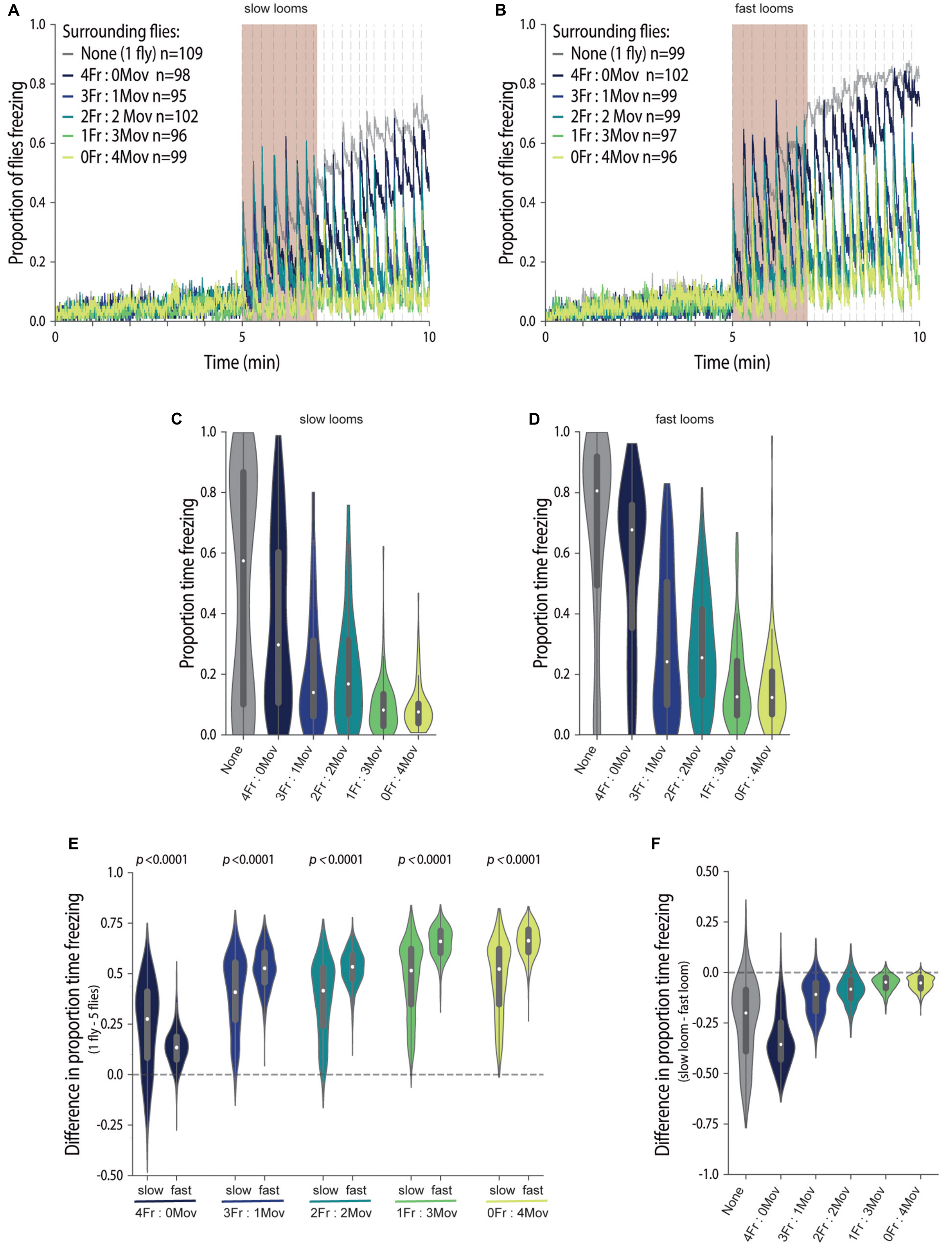

FIGURE 4 | Social environment overrides the perceived imminence of a threat in guiding freezing responses. **(A–F)** We manipulated focal flies to surround with four flies creating groups with different proportions of flies that always move (blind flies, NorpA) and flies that are optogenetically made to freeze (LC6 > CsChrimson). The colours codes for group composition are presented in **(A,B)**. Fraction of focal flies freezing throughout the experiment when exposed to slow **(A)** or fast looming

(Continued)

FIGURE 4 | stimuli **(B)**; dashed lines represent looming stimuli presentations; *n* represents the numbers of flies tested for each condition. **(C–F)** Violin plots representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: central white dot, median; [box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. Proportion of time spent freezing in the stimulation period when exposed to slow **(C)** and fast **(D)** looms. **(E)** Difference in the proportion of time spent freezing between individually tested flies and flies tested in groups for slow and fast looms (refer to the section "Materials and Methods." *P*-values result from the two-tailed Mann–Whitney test. **(F)** Difference in the proportion of time spent freezing between focal flies exposed to the two looming speeds, for each group composition (refer to the section "Materials and Methods"). Statistical comparisons between conditions are presented in **Supplementary Table 1**.

the induction of jumps, which affects freezing entries as mentioned above.

Importantly, we could now compare the impact of similar social cues across different threat levels, by plotting the difference between freezing by focal flies tested individually and in each social condition, for both fast and slow looms (refer to the section "Materials and Methods"; **Figure 4E**). We found that the impact of the social environment was stronger when flies were exposed to the faster loom in the presence of moving flies, which provide social cues of safety, as the differences relative to individually tested flies were bigger for flies exposed to fast than for flies exposed to slow looms (MW $p < 0.0001$; **Figure 4E**).

As mentioned above, freezing responses scale with threat imminence, but the social environment seems to have a bigger weight in the presence of a higher threat level, which may result from stronger impact of the social safety cues. Indeed, when comparing the difference in freezing responses between looming speeds, across the graded social motion cues, it is evident that there is an effect of looming speed on the time spent freezing (refer to the section "Materials and Methods"; KW $p < 0.0001$, statistic = 2,419; **Figure 4F** and **Supplementary Table 1**), but that this effect decreases and flattens out with the addition of moving flies to the social environment [Prob(Fexit) = 0.11–0.079 IQR −0.19 to 0.26, **Figure 4F** and **Supplementary Table 1**]. To summarize, adding moving flies, hence adding motion cues, levels out differences in freezing responses across looming speeds.

To further understand the effect of these tightly controlled social cues on freezing responses in groups, we once again focused on freezing exits in between loom presentations (**Figure 5**). Overall, for both looming speeds, increasingly adding motion cues, by adding moving flies, leads to an increase in the probability of freezing exit (KW $p < 0.0001$, statistic = 140.4, **Figure 5A**; KW $p < 0.0001$, statistic = 306.1, **Figure 5B**). The stronger impact of social cues of safety on the responses to faster looms is again evident comparing the magnitude of the difference between freezing exits by focal flies tested individually and in the presence of moving flies for both fast and slow looms (MW $p < 0.0001$; **Figure 5C**). In addition, although there are differences in the probability of freezing exit across looming speeds for all group compositions, with slower looms inducing higher freezing exit probabilities, these differences once again become very small as soon as one moving fly is added [Prob(Fexit) = 0.12–0.033 IQR −0.012 to 0.17, **Figure 5D** and **Supplementary Table 2**].

The finding that flies exposed to strong motion safety cues show decreased total time spent freezing and increased probability of freezing exits, for both loom speeds, indicates that the length of individual freezing bouts is decreased. Indeed, for all social conditions, freezing bouts that end in a freezing break between looms are shorter when more moving flies are around (**Figures 5E,F**), and hence, the bout length depends on stimulus strength, i.e., the level of surrounding motion cues. This suggests that flies gather information about safety for longer periods of time, when this information is sparser. Interestingly, at all stimulus (motion cue) strength levels, the distribution of these freezing bout lengths exhibited positive skew, a characteristic of information accumulation over time until the animal makes a decision (Luce, 1986; Ratcliff and Smith, 2004; Gold and Shadlen, 2007; Carandini and Churchland, 2013). Interestingly, social cues of danger provided by four freezing flies seem to be more salient for flies exposed to faster looms (**Figures 5C,D**); however, an appropriate analysis of this effect warrants a different experimental design in which jumps do not confound the analysis. In conclusion, social cues of safety lead to similar probabilities of exiting freezing across looming speeds and hence override differences in threat level.

DISCUSSION

In this study, we have addressed how threat imminence impacts defensive behaviors in groups and reliance on social cues. We showed that flies respond with different freezing levels to looming stimuli approaching at different speeds, whether tested individually or in groups, with faster looms triggering faster and more sustained freezing responses. Interestingly, we identified a non-linear scaling effect of looming speed, that is, the increase in freezing caused by exposure to a higher threat is not similar across social conditions. This increase in freezing was more pronounced for flies tested individually than flies tested in groups, indicating that the groups of flies exposed to faster looms showed a stronger social buffering effect than the groups exposed to slower looms. Moreover, controlling the social cues surrounding a fly through the manipulation of group composition under both looming conditions revealed that social cues of safety override differences in freezing responses to the two threat levels.

With the manipulation of looming speeds, we observed that faster looms lead to a faster engagement in freezing responses, which are then maintained for more prolonged periods of time, whether flies are tested individually or in groups. These findings are consistent with a perceived higher threat level for faster looms leading to a more vigorous response whose intended outcome is undetectability by a potential predator until safety is established. On the one hand, the differences between the latencies to start freezing are in line with differences in latencies to escape in fish (Bhattacharyya et al., 2017). On the other hand, freezing

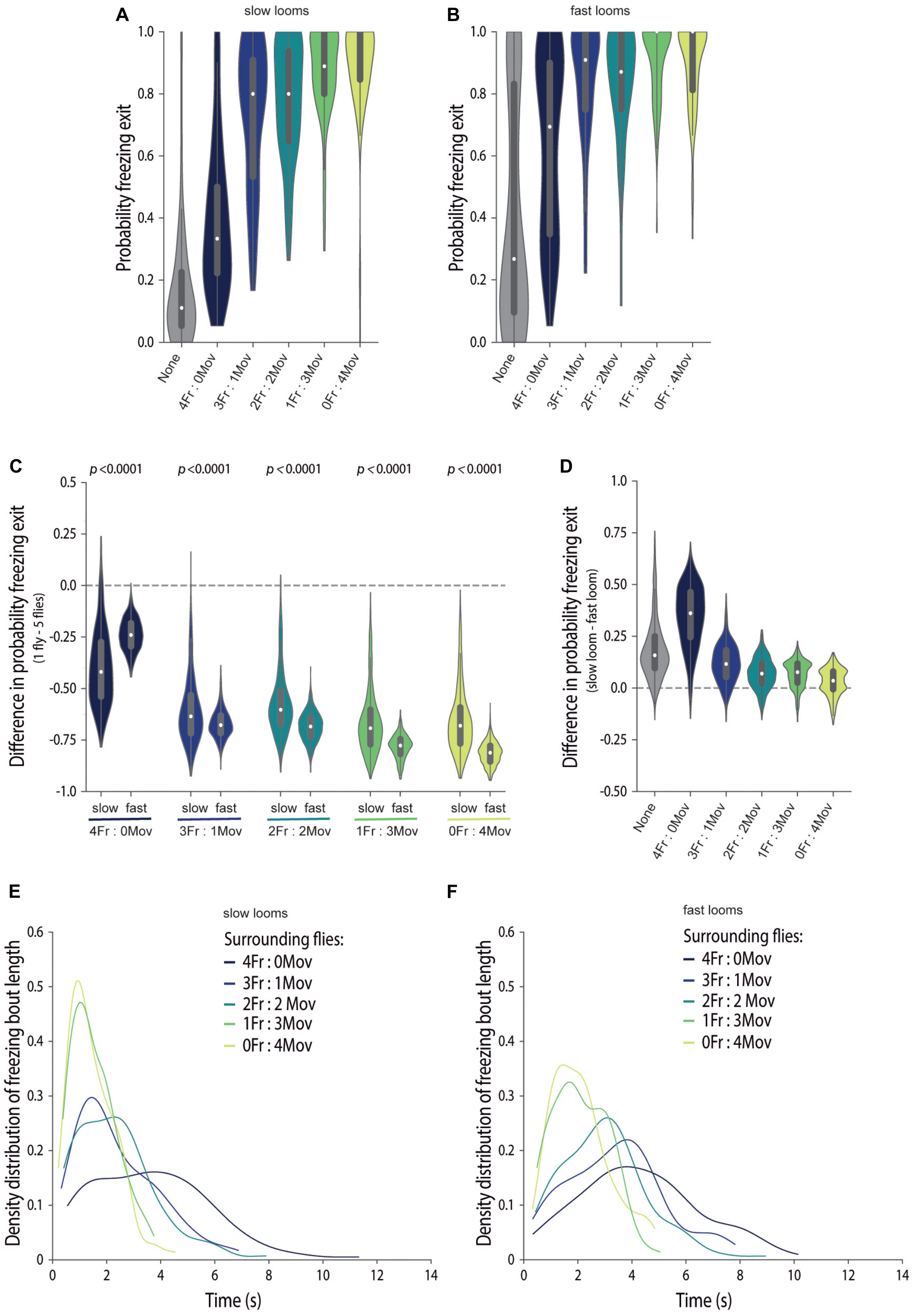

FIGURE 5 | Social cues of safety lead to freezing breaks, which underlie freezing response similarities across threat imminences. **(A–F)** We manipulated focal flies to surround with four flies creating groups with different proportions of flies that always move (blind flies, NorpA) and flies that are optogenetically made to freeze (LC6 > CsChrimson). The color code for the groups is presented in **(E,F)**; gray shadings represent an individually tested wild-type fly. **(A–D)** Violin plots representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: central white dot, median; [box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. Probability of freezing exit before the following looming stimulus when exposed to slow **(A)** or fast looming stimuli **(B)**. **(C)** Difference in the probability of freezing exit between individually tested flies and flies tested in groups for slow and fast looms (refer to the section "Materials and Methods"). *P*-values result from the two-tailed Mann–Whitney test. **(D)** Difference in the probability of freezing exit between focal flies exposed to the two looming speeds, for each group composition (refer to the section "Materials and Methods"). Statistical comparisons between conditions are presented in **Supplementary Table 2**. Kernel density estimate plots of the distribution of freezing bout lengths for flies exposed to slow **(E)** and fast **(F)** looms.

duration increases with faster looming speeds at an apparent contrast with the reported shorter escape duration for fast looms. However, a closer examination of the flies' behavior suggests that at a functional level, the change is in the direction of increased protection. Flies exposed to an escapable fast loom cut short their sequence of preparatory behaviors that ensures a controlled take-off flight away from the predator, resulting in a faster take-off, albeit less controlled. The small difference in take-off duration may grant precious time to flies to survive the chase (von Reyn et al., 2014). When exposed to an inescapable fast loom, flies freeze for a longer period of time, which may allow them to remain undetected in case the predator looms again in a second chase attempt.

Crucially, we identified that a graded manipulation of the social environment, providing graded levels of motion cues, induces graded freezing responses. Furthermore, underlying the graded amount of total time spent freezing is the modulation of the probability of freezing exit, resulting in graded freezing bout durations. These freezing bout durations uncover progressively faster freezing disengagement, that is, decreasing reaction times to increasing safety motion cues, for both threat imminences. With these findings, we uncovered a different strategy to that observed in social copying in the context of reproduction-related decisions in flies (Danchin et al., 2018), where animals adopt a conformity strategy, following the decision of the majority of others. If flies in our experiments were conforming to the majority, one would expect higher freezing levels for groups with 3 and 4 freezing flies, which was not the case. The finding that flies in our experimental conditions do not conform to the group majority and show stronger social buffering when exposed to a higher threat level may seem surprising. It is possible, however, that very sustained freezing responses, several minutes at a time, may become too expensive (Barrios et al., 2021) and that responding to the social environment may reduce the cost without significantly reducing the flies' defenses. The behavioral pattern we observed is consistent with such a strategy, as flies responded to the looming stimulus with freezing even in groups with moving flies; however, each incremental addition of social safety cues lead them to disengage from freezing after increasingly shorter times. This pattern is reminiscent of a process of evidence integration, of safety cues, to decision bound–resumption of activity. A finer grained investigation of how freezing responses of individuals and flies in groups vary with threat level, with careful control and monitoring of motion cues, will permit determining whether freezing responses in flies follow an integrate to threshold model of decision making, analogously to that observed for escape decisions in mice (Evans et al., 2018) and two-alternative forced choices visual and olfactory tasks in primates, rodents, and flies (Luce, 1986; Ratcliff and Smith, 2004; Gold and Shadlen, 2007; Carandini and Churchland, 2013; DasGupta et al., 2014).

Importantly, we uncovered a hitherto unknown, non-linear scaling effect of defense responses in groups with threat imminence. The enhanced social buffering effect upon fast looms seems to result from the perception of the approach speed of the threat in interaction with the perception of surrounding social cues, raising the question of whether this effect is generalizable across different features of threat that convey different degrees of danger, such as contrast of the threatening stimulus relative to the background, or rather specific to approach speed. To address this issue, it will be interesting to analyze freezing responses tampering with various features of predation threat.

Interestingly, social cues of danger produced by surrounding freezing flies seem to exacerbate freezing responses to the faster loom compared with the slower loom, as the former are a lot less likely to exit freezing. However, our experiments do not allow addressing the social effect on freezing onset appropriately as the optogenetic manipulations used also produce strong jumping responses. Future experiments inducing freezing without jumps will allow studying the interplay between threat levels and social cues of danger.

We believe that this study opens up a path to understand the dynamics of the usage of individually percieved data about threat and danger cues in different predation settings, which will provide valuable insight into how crucial threat response decisions are made.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available at doi: 10.6084/m9.figshare.19609782.

AUTHOR CONTRIBUTIONS

CF and MM conceived the project and designed the experiments with input from MH. CF and MH performed all experiments except for optogenetic activation in the absence of looming, which was done by MF. RG made fly crosses and prepared flies for the experiments. MF updated behavioral setups and video acquisition codes. MF and MH wrote tracking and behavior classification codes. CF, MH, and MF wrote the analysis code. CF and MH analyzed the data. CF, MH, and MM discussed the results. CF and MM wrote the manuscript. All authors commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.885795/full#supplementary-material

Supplementary Figure 1 | Effect of looming speed on latency to freezing onset and freezing bout length. Distribution of the time points of freezing onset after looming for flies tested individually **(A)** and in groups **(B)**. Dashed gray lines represent looming onset and offset. Cumulative distributions of freezing bout lengths for flies tested individually **(C)** and in groups **(D)**. Orange denotes flies exposed to slow looms and green denotes flies exposed to fast looms.

Supplementary Figure 2 | Motion cues in groups scale with threat intensity. Violin plots representing the probability density distribution of individual fly data bound to the range of possible values, with boxplots elements: [central white dot, median; box limits, upper (75) and lower (25) quartiles; whiskers, 1.5 × interquartile range]. Average motion cues a focal fly is exposed to the stimulation period. *P*-value results from the two-tailed Mann–Whitney test.

Supplementary Figure 3 | Freezing and jumping responses of optogenetically activated LC6 > CsChrimson. **(A)** Fraction of flies freezing throughout the experiment while providing pulsed red light at the timestamps normally used to provide looming stimuli (dashed lines); LC6 > CsChrimson flies supplemented with retinal (blue) and control without (gray). **(B–D)** We manipulated four out of the five flies in group, to surround focal flies with groups with different proportions of flies that always move (blind flies, NorpA) and flies that are optogenetically made to freeze (LC6 > CsChrimson), while presenting looming stimuli. The color code for the groups is presented in **(B–D)**. Fraction of surrounding, manipulated, flies freezing throughout the experiment when exposed to slow **(B)** or fast looming stimuli **(C)**; dashed lines represent looming stimuli presentations. **(D,E)** Data from flies exposed to slow looms. **(D)** Number of jumps throughout the experiment by the surrounding manipulated flies. **(E)** Number of jumps at the first loom presentation for LC6 > CsChrimson flies in groups of four surrounding optogenetically manipulated flies.

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Did the transition to complex societies in the Holocene drive a reduction in brain size? A reassessment of the DeSilva et al. (2021) hypothesis

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Introduction

Encephalization has long been understood to be a key adaptation in the human lineage, and over the last four million years species attributed to *Australopithecus* and *Homo* have shown demonstrable trends toward increased brain size. However, our understanding of past populations is limited by our reliance on the fossil record. For some poorly preserved species, we are currently dependent on a few or even a single cranium. This places limits on our ability to infer subtle changes in brain size, even as the broader trend of encephalization is clear.

DeSilva et al. (2021) hypothesize that modern human brain size has decreased, starting at roughly 3,000 years ago. They offer a model in which directional selection for decreased brain size, and/or stabilizing selection for maintaining large brains, was relaxed due to the ability to store information externally in social groups. Under this model, which they analogize from ants, following the development of complex societies, the cumulative intelligence and knowledge of the social group acted to relax the strong forces of selection that had been present in earlier human populations. They propose that "group-level cognition may select for reduced brain size and/or adaptive brain size variation" (DeSilva et al., 2021, p 7).

Do we see a decrease in brain size in modern humans?

DeSilva et al. (2021) base their relevant conclusions on the results of a "change-point analysis," in which they identify a change in mean brain size, starting at roughly 3,000 years ago (or 3 ka), coinciding with the widespread appearance of complex societies. Their data set is a collection of 987 fossil and museum specimens ranging from Miocene hominid *Rudapithecus* (9.85 million years ago or Ma) to modern humans (300 ka to 100 years).

The dataset itself raises several questions. The first is whether the sample is appropriate to test the hypothesis that brain size reduced due to changes associated with the transition to agriculture and the rise of complex societies. Since this transition occurred at different times across the globe (see Barker, 2009), rather than over a single 3–5 ka year period, under the hypothesis of DeSilva et al. (2021) we should detect the change in different modern human populations at different times. However, the dataset of DeSilva et al. (2021) is not organized to test the hypothesis in this fashion. Populations from around the globe are lumped together, with only 23 crania sampled over what we would argue to be a critical window with regards to their hypothesis, 5–1 ka, and coming from Algeria, England, Mali, China, and Kenya, among other locations. Later modern human samples are focused on Zimbabwe (at 1.06 ka), the Pecos Pueblo sample from the United States (1 ka), and finally, 165 crania (28% of the total sample) are from Australian pre-Neolithic hunter-gatherer populations and dated in DeSilva et al. (2021) to 100 years ago. In that same dating category, 307 (53% of the total sample) are from unspecified Morton Collection crania, where we have no way of knowing how many may be from pre-Neolithic and post-Neolithic populations. We also observe that the sample of DeSilva et al. (2021) generates a modern human mean of 1,297 cc in the final 100-year category, which is well below other published estimates of contemporary world-wide modern mean human cranial capacity that range from ∼1,340 cc up to ∼1,460 cc (Beals et al., 1984; Henneberg, 1988; Ruff et al., 1997; DeSousa and Cunha, 2012).

Second, the sample relies on the combination of fossil and modern human specimens, with temporal intervals between these crania ranging from 2.85 million years (Ma) to 100 years, and data points at each time interval ranging from 578 to 1 (Figure 1). Even within their sample of modern humans, starting with the 300-ka year old Jebel Irhoud skulls from Morocco, the intervals range from 105 ka years to 100 years. This produces a heavily unbalanced sample. Of the 987 total specimens (and 836 modern human crania) in the analysis, 578 specimens are from just two sources—the Morton Collection in Philadelphia and Peter Brown's Analysis of Australian and Asian recent modern humans (Brown et al., 2004; Lewis et al., 2011). Both samples are placed in the final 100-years category, which means that more than half of the specimens of a 9.8-million-year analysis are placed in the final 100 years (Figure 1).

Change-point analysis is, broadly speaking, a form of regression in which the hypothesis being tested is whether there is a change in the distribution of a particular parameter (e.g., slope, residual variance, mean, etc.; Chow, 1960; Chen and Gupta, 2012). This type of analysis relies on the standard assumptions of regression: that the residuals do not deviate from (multivariate) normality and homoscedasticity. However, in the DeSilva et al. (2021) data set, the extremely disproportionate sampling of more recent populations leads to violations of these assumptions. Using the full dataset, the residuals from the change-point analysis model (Figure 1) are significantly skewed (Mardia's test $p < 0.00001$) and, depending on the statistic, approaching heteroscedastity (Breusch-Pagan $p <= 0.100$, White test $p = 0.059$). Even if the data are reduced to just the modern human data set starting at ∼300 ka—to test the hypothesis of micro-evolution within modern humans—the residuals are still heavily skewed (Mardia's test $p < 0.00001$).

To understand relevant patterns over time, we propose that several adjustments must be made to the data. First, we limited the analysis to modern humans only. We challenge the notion that brain size changes between such adaptively divergent and temporally distant groups such as *Rudapithecus, Australopithecus,* and *Homo erectus* in any way inform on hypotheses relating to potential changes in modern human brain size around 3 ka driven by transitions to agriculture and social complexity in the Holocene. Analyses must be done at the appropriate scale (Du et al., 2018), and hypotheses of micro-evolutionary change within species should be addressed with data at the appropriate micro-evolutionary scale. In addition, ignoring interspecific relationships (i.e., phylogeny) and population structure can have unforeseen consequences on analyses (e.g., Felsenstein, 1985; Roseman and Auerbach, 2015). Thus, we limited the data to only modern humans changing the analysis to focus on only the last 300 ka. While including modern human population structure in our analysis (e.g., Roseman and Auerbach, 2015) could be warranted, this would require a completely different approach than used in DeSilva et al. (2021) and here we merely wish to explore the effect of using their same methods on data that is more appropriate to the research question.

Second, we reduced the data to means calculated to represent specific temporal slices, following on standard practice in time-series analysis (e.g., Auger and Lawrence, 1989; Wagner et al., 2002; Lopez et al., 2010; Palma, 2016; Hites, 2019). Using the data from DeSilva et al. (2021), we calculated 100-year mean values for the modern human data set, which focuses on the last 300 ka (Figure 2).

However, due to of the paucity of older samples, even within the single-species modern human sample, these data were still significantly skewed (Mardia's test $p < 0.0001$). Because 60% of the data fall within the last 10% of the modern human timeline, our final step was to create a subset of the data that encompassed the data over this most well-populated interval, the last 30 ka (Figure 3). This created a more normally distributed time series, (Mardia's test $p = 0.956$), with the largest temporal gap reduced to only 2.8 ka. We suggest that, given the data available from DeSilva et al. (2021), this consolidated and narrowed data set is needed to produce an accurate test of the hypothesis of a shift in mean brain size in modern humans in the pre and post-agricultural period.

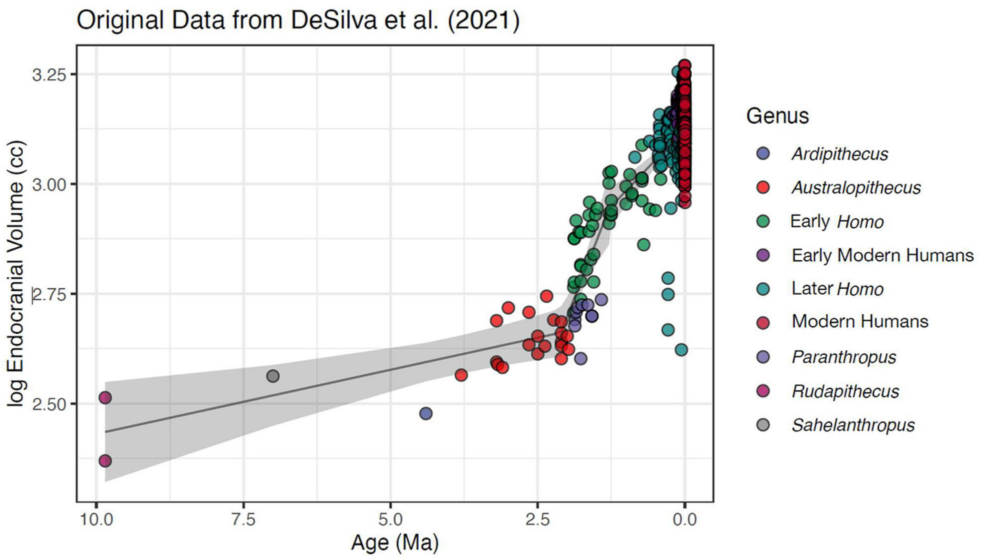

FIGURE 1
Original dataset from DeSilva et al. (2021). Residuals from the regression are heavily skewed and heteroscedastic, and the temporal intervals between samples ranges from 2.85 million years to 100 years. Of the total of 987 total specimens in this 9.85 Ma analysis, 578 are in the final 100-year interval. Also shown is fitted regression lines from a linear model with 95% confidence intervals around the slope; changepoints were found using *segmented* R package at 2.1 ± 0.1 Ma, 1.3 ± 0.1 Ma, and respective slopes of 0.03 ± 0.01, 0.35 ± 0.04, 0.14 ± 0.13 surrounding changepoints. No changepoint was located at or around 3 ka that approached significance using Davies (1987) test after accounting for previously mentioned changepoints (p-value of any additional changepoints = 0.621).

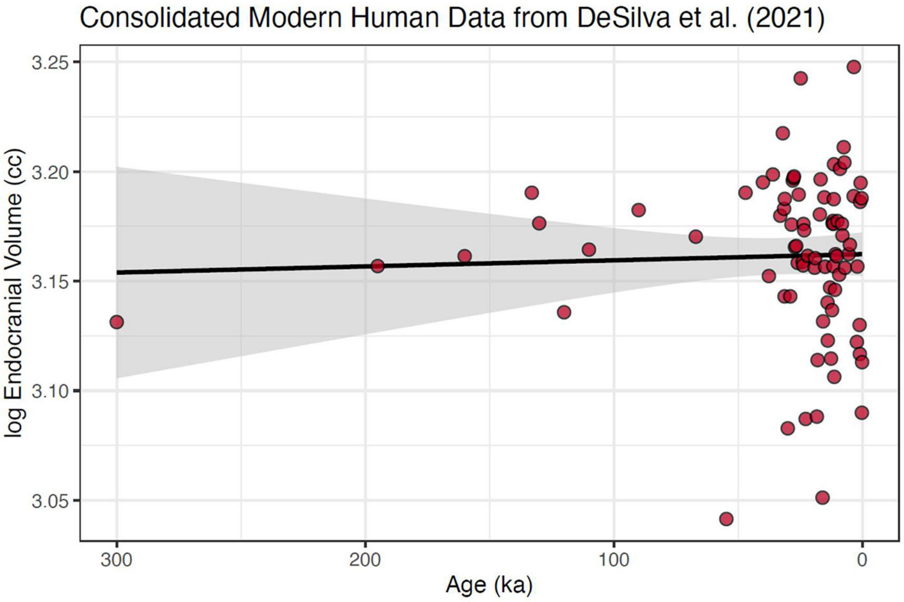

FIGURE 2
Modern human cranial size over the last 300 ka using data consolidated into 100-year means. This data set follows standard practice in time series analysis by having single values at each time slice. Further, the heteroscedasticity is strongly reduced (although it is still heavily skewed). Also shown is fitted regression line with 95% confidence interval around the slope; slope = $2.78e^{-5} \pm 8.89e^{-5}$, which is not significantly different from 0 with a p-value of 0.754. No changepoint was found for this consolidated and reduced dataset.

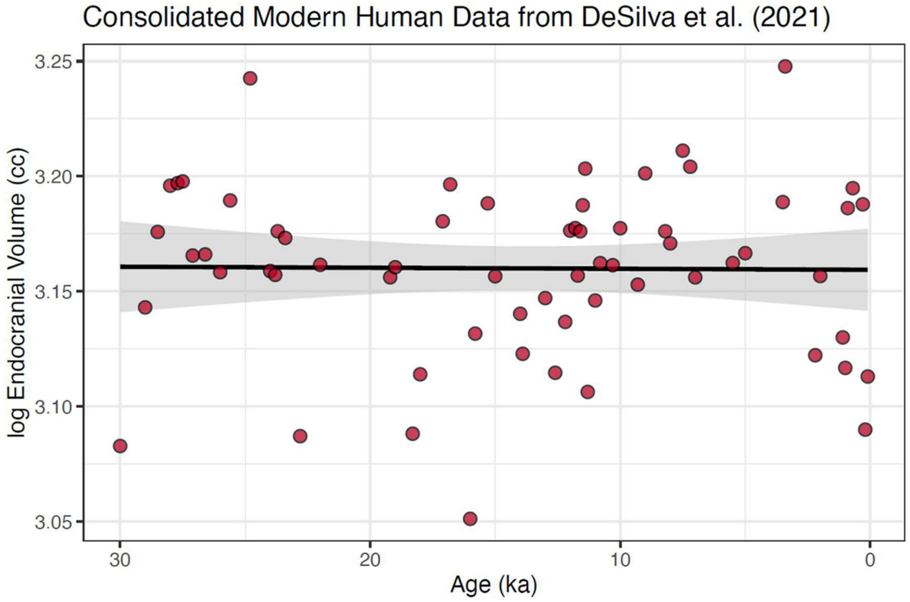

FIGURE 3
Modern human cranial size over the last 30 ka using data consolidated into 100-year means. This data set is neither heteroscedastic nor skewed. Further, we argue that this is the appropriate scale at which to examine the micro-evolutionary hypothesis of change due to selection changes in the Holocene. Also shown is fitted regression line with 95% confidence interval around the slope; slope = $2.80e^{-5} \pm 8.89e^{-5}$, which is not significantly different from 0 with a p-value of 0.754. No changepoint was found for this consolidated and reduced dataset.

There are multiple methods for identifying changepoints in series data (Chen and Gupta, 2012), and we employed two methods: using standard segmented regression in *SegReg* (www.waterlog.info) and the R Package *segmented* (Muggeo, 2008) to determine if there were shifts in slope following on the changepoint hypotheses of DeSilva et al. (2021). We first used our consolidated modern human 300 ka dataset, and then the consolidated modern human 30 ka dataset.

Using either data set and either software packages, we found no significant changepoint at or near 3 ka (Figures 2, 3). This includes using the same R packages and data as in DeSilva et al. (2021), with the addition of a Davies (1987) test for significance of a changepoint around 3 ka in the package *segmented* (Muggeo, 2008), a step DeSilva et al. (2021) did not report (p-value for any changepoints in the consolidated 300 ka = 0.739; and p-value for any in the consolidated 30 ka dataset = 0.259).

The Davies test (Davies, 1987) is necessary in cases where a new parameter enters the model under the alternative hypothesis, here testing if the difference between the slopes at the changepoint is significantly different from zero vs. a null hypothesis of no difference as the null hypothesis has one less parameter—the changepoint (Muggeo, 2008). Calculating 100-year means on the modern human data had the effect of reducing the residuals heteroscedasticity for the 300 ka dataset (Breusch-Pagan p = 0.501, White Test p = 0.538), and for the 30 ka dataset (Breusch-Pagan p =0.610, White Test p = 0.877).

With regards to testing the hypothesis in question, our analyses showed no changes in brain size associated with the transition to agriculture during the Holocene. Overall, our conclusion is that, given a dataset more appropriate to the research question, human brain size has been remarkably stable over the last 300 ka. Thus, hypotheses of recent change are not supported by the evidence (see also Beals et al., 1984; Henneberg, 1988; Ruff et al., 1997; DeSilva et al., 2021).

Discussion

DeSilva et al. (2021) propose that human brain size has decreased, and offer innovative reasons why this may be so, primarily focusing on a model of “group level cognition.” Our analysis of these data fails to find a decrease in human brain size over the last few thousands of years. When the large sample sizes of the most recent human samples are adjusted for, the pattern disappears, and the arguments no longer need to be invoked.

We argue that, when examining questions of micro-evolutionary change, the analysis and data need to be appropriate for the specific scale of that hypothesis. Further, the data need to be otherwise relevant for the hypothesis being tested (see Houle et al., 2011). Given that the adoption of agriculture and the transition to complex societies occurred in different times at different places, the samples need to be specific enough to test the hypothesis across different times and populations, which does not appear to be the case in this instance.

Author contributions

BV and MG analyzed data and wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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What is really social about social insect cognition?

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It is often assumed that social life imposes specific cognitive demands for animals to communicate, cooperate and compete, ultimately requiring larger brains. The “social brain” hypothesis is supported by data in primates and some other vertebrates, but doubts have been raised over its applicability to other taxa, and in particular insects. Here, we review recent advances in insect cognition research and ask whether we can identify cognitive capacities that are specific to social species. One difficulty involved in testing the social brain hypothesis in insects is that many of the model species used in cognition studies are highly social (eusocial), and comparatively little work has been done in insects that live in less integrated social structures or that are solitary. As more species are studied, it is becoming clear that insects share a rich cognitive repertoire and that these abilities are not directly related to their level of social complexity. Moreover, some of the cognitive mechanisms involved in many social interactions may not differ from those involved in non-social behaviors. We discuss the need for a more comparative and neurobiologically grounded research agenda to better understand the evolution of insect brains and cognition.

KEYWORDS

social brain hypothesis, insects, social evolution, comparative cognition, foraging, mate competition

1. Introduction

The considerable variation in brain size and cognitive abilities in the animal kingdom has been a long-standing question for biologists (Healy, 2021). It is broadly accepted that the brain evolved to process ecological information. However, it is an energetically expensive organ to operate and maintain. Therefore explanations for its evolution must reconcile these costs with fitness payoffs (Aiello and Wheeler, 1995). This presented an especially intriguing puzzle for primatologists who observed that the brains of primates were significantly larger than predicted, given their body size (Clutton-Brock and Harvey, 1980), and that classical explanations implicating their need for complex problem-solving (Gibson, 1986) did not account for these trends in all primate groups. The correlation between neocortex size and social group size in primates hinted at a link between brain size and sociality, later formalized as the “social brain hypothesis” (Dunbar, 1992; Dunbar, 1998). Following this hypothesis, increases in the frequency and complexity of social interactions, and the cognitive challenges of managing social relationships, may have required better information processing capabilities and larger brains. Over the past decades, research in primates has yielded experimental support for this hypothesis, reporting that neocortex size is also associated with intensity of reproductive competition (Lindenfors, 2005), frequency of coalition formation (Dunbar and Shultz, 2007), social play (Lewis, 2001), and social learning (Reader and Laland, 2002).

The story is a bit different in insects. While early description of insect anatomy and behavior also suggested a strong relationship between cognition and sociality (Dujardin, 1850), recent attempts to correlate brain size with metrics of social complexity are ambiguous (Riveros et al., 2012; O'Donnell et al., 2015; Farris, 2016; Kamhi et al., 2016; Gordon et al., 2019; Kamhi et al., 2019), emphasizing the need for more research (Lihoreau et al., 2012a; Simons and Tibbetts, 2019). Insects, like primates, display diverse social forms (Costa, 2006; Hölldobler and Wilson, 2009) which are accompanied by brain size variations (Strausfeld, 2012). However, the social organization of insect colonies can be dramatically different from that of vertebrates. In their most socially advanced forms (eusociality), insect colonies are characterized by extensive division of labor, cooperative brood rearing, and overlapping generations of adults (Batra, 1966; Michener, 1974; Wilson, 1975). This means that workers (i.e., the individuals on which most cognitive studies are performed) are sterile and fitness payoffs are experienced at the level of the group. Reproductive competition, mate selection and pair bonding - some of the primary drivers of brain evolution in social vertebrates (Healy, 2021) - therefore do not occur in these individuals. In fact, increases in insect social complexity are predicted to decrease overall brain size through obligate division of labor which allows for investment in functionally specialized brain regions (Gronenberg and Riveros, 2009; O'Donnell et al., 2015). In this way, the social structure of an insect colony may reduce the need for individual investment in neural tissue while improving overall performance in a few specialized tasks.

These recent theoretical developments, coupled with the observation that most of the impressive cognitive feats reported in insects have been identified in the context of individual foraging, and not social interactions (von Frisch, 1967; Giurfa, 2013; Wehner, 2020), draw attention to the emerging role of competition and foraging ecology in the evolution of insect cognition (Kamhi et al., 2016, 2019). For instance, a phylogenetic analysis has shown that the elaborated mushroom bodies in the insect brain (i.e., neuroanatomical structures involved in learning and memory), were acquired roughly 90MYR before the evolution of eusociality, coinciding with the switch from phytophagy to parasitoidism and the origin of central-place foraging (Farris and Schulmeister, 2011). This suggests the challenge of navigating and finding food in various types of landscapes drove the evolution of enhanced cognitive processes in what would later become eusocial insects (Jeanson and Weidenmüller, 2014; Farris, 2016). This observation thus raises the possibility that enhanced cognitive abilities may not be unique to just eusocial insects, but could be observed in any species that experiences the appropriate ecological conditions.

In this mini-review we consider whether many of the cognitive abilities once thought to be exclusive to social insects can evolve through non-social ecological processes. We argue that the primary drivers of insect cognition are processes related to reproductive competition and foraging. Using the extensive body of literature surrounding cognitive abilities in highly eusocial hymenopterans (i.e., honey bees, ants, and wasps) and the more recent, though rapidly expanding field of non-eusocial insect cognition (e.g., drosophila), we show that enhanced cognition abilities can evolve in other social structures (encompassing presocial, subsocial, semisocial, parasocial, quasisocial, facultatively social, cooperative breeding, gregarious, etc. species, *sensu* Michener, 1974; Wilson, 1975; Crespi and Yanega, 1995; Costa, 2006) and in solitary species.

2. Cognitive abilities shared by eusocial and non-eusocial insects

Advanced levels of sociality require cognitive abilities supporting precise social interactions (Byrne and Bates, 2007). For insects, this may mean being able to assess group identity, recognize specific individuals, remember past interactions with them, share information, find and learn the localization of food resources and navigate back to the colony nest (Wenseleers and van Zweden, 2017). Below we describe how many of these cognitive abilities, once thought to be unique to eusocial insects, have in fact recently been reported in some non-eusocial species and we discuss the possible explanations for how such traits could have evolved independently of sociality (see summary in Table 1).

2.1. Individual recognition

Targeted social interactions, such as cooperation, may require specific recognition systems to identify different types of individuals in a group or population (Anderson and McShea, 2001; Wenseleers and van Zweden, 2017). As such, eusocial insects have evolved recognition systems enabling them to discriminate nestmates and non-nestmates (van Zweden and d'Ettorre, 2010), as well as individuals from specific castes within the colony (van Oystaeyen et al., 2014), through the use of odor cues. In some small colonies where dominance hierarchies are established to determine reproductive priority, insects also possess the ability to recognize individual nestmates. This is the case of colonies founded by several reproductive females, like for instance the paper wasps *Polistes fuscatus* that have unique facial coloration patterns (Tibbetts, 2002), or the ants *Pachycondylla villosa* that carry individual chemical signatures (D'Ettorre and Heinze, 2005). Though these recognition mechanisms have been implicated in the ecological success of insect societies (Jeanson and Weidenmüller, 2014) they are not specific to eusocial species.

TABLE 1 Some examples of cognitive abilities reported in eusocial and non-eusocial insect species.

	Eusocial	Non-eusocial
Individual recognition	- Wasps establish reproductive dominance hierarchies based on facial coloration patterns (Tibbetts, 2002)	- Fruit flies establish dominance hierarchies for mate competition (Yurkovic et al., 2006) (unknown mechanism)
	- Ant queens establish reproductive dominance hierarchies based on chemical cues (D'Ettorre and Heinze, 2005)	- Crickets select mating partners through self-referent odor phenotype matching (Capodeanu-Nägler et al., 2014)
Numerosity	- Honey bees count landmarks (Chittka and Geiger, 1995), perform basic additions (Howard et al., 2019), and have the concept of zero (Howard et al., 2018)	- Tenebrio beetles adjust mate guarding duration to the number of males encountered before mating (Carazo et al., 2009)
	-Desert ants count steps to evaluate distances during path integration (Wittlinger et al., 2006)	- Ladybugs adjust egg laying strategy according to the number of larvae or other females encountered (Hemptinne et al., 1992)
Non-elemental learning	- Honey bees solve reversal learning and patterning tasks (Boitard et al., 2015; Devaud et al., 2015)	- Fruit flies solve reversal learning (Mancini et al., 2019) and patterning tasks (Durrieu et al., 2020)
Social learning	- Honey bees share information locational information about food sources (von Frisch, 1967) through the waggle dance	- Fruit flies copy the choices of oviposition sites (Battesti et al., 2012) and mating partners (Danchin et al., 2018) through visual observation
	- Bumblebee bees learn flower preferences (Leadbeater and Chittka, 2005; Worden and papaj, 2005) and new foraging techniques (Alem et al., 2016; Loukola et al., 2017) through visual observation	
Tool use	- Some ant species use soil debris to soak liquid food and carry it back to their nest (Maak et al., 2020)	-Ant-lion larvae throw sand to make their prey fall into the bottom of their pit (Oguma, 1930)
	- Several ant and wasp species close their nest using soil/stone deposits (Pierce, 1986)	-African tree crickets use and manipulate leaves to amplify their calls (Prozesky-Schulze et al., 1975)
		- Solitary wasps use pebbles to compact soil to close their burrow (Brockmann, 1985)
Emotions	- Honey bees show a pessimistic judgment bias after stress in their expectation of a food reward (Bateson et al., 2011)	- Fruit flies show a pessimistic judgment bias after stress in their expectation of a food reward vs. punishment (Deakin et al., 2018)
	- Bumblebees show optimistic judgment biases after an unexpected food reward (Perry et al., 2016)	
Navigation	- Ants and bees learn places (von Frisch, 1967; Wehner, 2020)	- Fruit flies and solitary wasps learn places (Tinbergen, 1932; Ofstad et al., 2011)
	- Ants and bees learn routes between their nest and a goal (von Frisch, 1967; Wehner, 2020)	- Dung beetles learn routes through celestial cues and wind compass, and path integration (Dacke et al., 2019)
	- Honey bees and bumblebees develop traplines to exploit multiple feeding sites (Lihoreau et al., 2012; Buatois and Lihoreau, 2016)	- Butterflies use traplines (Young and Montgomery, 2020)

Theoretical models predict that the capacity to recognize group members should peak at intermediate levels of sociality and decrease as societies become larger (Gronenberg and Riveros, 2009), possibly due to the inability to manage so many social interactions and the risk of genetic conflicts in large colonies with different patrilines (Ratnieks et al., 2006). Moreover, if cognitive processes involved in individual recognition evolved due to the demands of recognizing reproductive competitors (Tibbetts, 2002), insect societies composed of highly related individuals should not possess such abilities. In line with these predictions, recent work shows males of the fruit fly *Drosophila melanogaster*, which form temporary aggregations on food sources for feeding and mating, appear to recognize other males with whom they compete for access to females (Yurkovic et al., 2006), although the cues involved are still unknown. In the solitary decorated cricket (*Gryllodes sigillatus*), individuals acquire the scent of their partners after mating. Female crickets, who recognize their own odor through self-referent phenotype matching favour pairings with males with unfamiliar odors to maximize their number of mating partners (Capodeanu-Nägler et al., 2014). While more studies are needed to better understand the evolution of individual recognition across insects, these observations in eusocial and non-eusocial species suggest individual recognition may be more closely related to reproductive competition and mate selection, rather than just social life.

2.2. Numerosity

Social life may require the ability for individuals to assess quantities and estimate the number of social partners or potential competitors in a group (Rios and Kraenkel, 2017). Several studies suggest eusocial honey bees (*Apis mellifera*) can count landmarks (Chittka and Geiger, 1995), recognize the number of objects in an image (Gross et al., 2009), perform basic operations (Howard et al., 2019), and understand the concept of zero (Howard et al., 2018, but see MaBouDi et al., 2021). Desert ants (*Cataglyphis fortis*) seem to count steps to evaluate distances during path integration (Wittlinger et al., 2006). However, whether these abilities are linked to sociality is not clear as many other behaviors could also require numerosity, like monitoring brood and forager populations and increasing resource collection to feed a colony, may simply result from individual variability in response threshold to environmental stimuli (i.e., the presence of pheromones, empty brood cells) not numeric competency (Page and Erber, 2002). Moreover, eusocial insect colonies can be so large so as to make recognizing and tracking all social interactions between conspecifics very unlikely (Gronenberg and Riveros, 2009). Thus, we would expect cognitive traits related to numerosity to evolve when monitoring potential rivals or mates is both feasible, given colony/population size, and is associated with increased reproductive fitness. Accordingly, some studies suggest non-eusocial insects have some sense of numerosity. For instance, males of the gregarious beetle *Tenebrio molitor* are capable of adjusting the intensity of their mate guarding strategy according to the number of males encountered before mating (Carazo et al., 2009). In the ladybug *Adalia bipunctata* females reduce egg laying according to the number of larvae from other females already encountered (Hemptinne et al., 1992). These observations, though relatively rare, indicate that insect numerosity may be more closely related to the likelihood of encountering competitors and mates than just group size.

2.3. Non-elemental learning

Living in a group may also multiply the need for animals to develop different types of associative learning. As more individuals are encountered, more types of interactions, relationships, and common experiences may have to be stored in memories (Bond et al., 2003). Honey bees (*A. mellifera*), that live in colonies with thousands of workers, can learn many elemental associations between a stimulus and a reward, but also more sophisticated non-elemental associations involving ambiguous stimuli (Giurfa, 2013). For instance, bees can learn to respond to a reinforced odor A and not to a non-reinforced odor B, and then have to learn the opposite when stimulus contingencies are reversed (Boitard et al., 2015). Honey bees can also be trained to patterning problems, by associating a simple component (A or B) to a reward and the mixture (AB) to an absence of reward or vice versa (Devaud et al., 2015). So far, however all these experiments simulate learning in a foraging context, suggesting that this might be a stronger driver of associative learning than social life *per se*. Accordingly, the gregarious fruit fly *D. melanogaster* was recently shown to solve similar non-elemental cognitive operations such as reversal learning (Mancini et al., 2019) and negative patterning (Durrieu et al., 2020), a task related to foraging for food or oviposition sites. Given these observations in non-eusocial insects, it might well be that these types of learning are primarily required to find food or mating partners rather than just for navigating more social interactions.

2.4. Social learning

Animals can learn about their environment by observing conspecifics (Laland, 2004). Information provided by others often improves individual decisions and is believed to be a major advantage of social life (Bourke, 2011). Famously, honey bees share information about the location of abundant food sources in the form of a symbolic waggle dance (von Frisch, 1967). Eusocial bumblebees can learn flower preferences through visual observation of more experienced conspecifics (Leadbeater and Chittka, 2005; Worden and Papaj, 2005) and new foraging techniques, such as pulling ropes (Alem et al., 2016) or rolling balls (Loukola et al., 2017). Interestingly, the study of the mechanisms underpinning flower choice copying in bumblebees showed this behavior can emerge from associative learning mechanisms that are not different from non-social learning (Dawson et al., 2013). It has thus been argued that social learning is just a form of individual learning with a social cue (Galef, 1995), and the emergence of social learning should correlate with the cost of information acquisition, not a direct result of group living. Supporting that theory, gregarious fruit flies *D. melanogaster* can also learn from their conspecifics. Females copy the choices of others for oviposition sites (Battesti et al., 2012) and mating partners (Danchin et al., 2018). Solitary wood crickets *Nemobius sylvestris* learn to hide after being in contact with conspecifics maintained under stressful conditions in the presence of spiders (Coolen et al., 2005). Thus, social learning and may employ associative mechanisms of non social learning can be observed in solitary species.

2.5. Tool use

In social groups with overlapping generations of individuals, social learning can lead to the emergence and cultural transmission of complex behaviors, like tool use (Shumaker et al., 2011). Such transmission is a form of heritable adaptation that is likely a key benefit of social life (Danchin et al., 2004). Several phenomena akin to tool use have been reported in eusocial insects (Pierce, 1986). For instance, workers of *Pogonomyrmex* and *Aphaenogaster* ant colonies occasionally use soil debris to transport liquid food that they could not otherwise bring back to their nests (Maak et al., 2020). However, examples of tool-users are also found in non-eusocial species. Antlion larvae throw sand to make their prey fall into the bottom of its pit (Oguma, 1930). African tree crickets pierce holes in leaves to create baffles and amplify their mating calls (Prozesky-Schulze et al., 1975). Solitary wasps use pebbles to compact the soil and close their burrows (Brockmann, 1985). Future research would benefit from identifying the specific learning mechanisms (i.e., social learning or trial and error) involved in tool use in these later examples remains unclear (Kenward et al., 2005). Once again, these abilities appear to be linked to foraging, sexual competition and defense, rather than the degree of sociality (Weir and Kacelnik, 2006).

2.6. Emotions

Many social interactions are regulated by emotional displays that enable communication between group members (van Kleef, 2009). In principle, being in a specific emotional state can improve responses to threats or social interactions and lead to more efficient collective responses. In this context, emotional states are elicited by rewarding and punishing goals, whereby rewards are stimuli that animals work to acquire and punishments are stimuli that they work to avoid Rolls (2014). Studies using judgment bias paradigms (Baciadonna and McElligott, 2015) indicate eusocial insects show emotional-like states. For instance, honey bees (*A. mellifera*) gently shaken just before the experiment showed pessimistic biases when faced the problem of classifying an ambiguous odor stimulus (Bateson et al., 2011) whereas bumblebees (*Bombus terrestris*) that received an unexpected reward showed consistent optimistic biases in a visual judgment task (Perry et al., 2016). However, similar emotional-like states were also recently reported in non-eusocial insects, such as in the gregarious fruit fly *D. melanogaster* where shaken flies showed a pessimistic bias in an ambiguous odor binary choice task, while control flies did not (Deakin et al., 2018). Again, this observation in non-eusocial fruit flies suggests emotional display is not specific to sociality. In fact, it is likely that these emotional states are triggered by basal neurohormonal mechanisms common across insect species (i.e., releases of biogenic amines) and increase efficiency of decision making in many contexts independent of sociality, such as foraging or predator avoidance.

2.7. Elaborate navigation

Social animals must intensely engage in foraging in order to gather and store large amounts of foods for their progeny. For central place foragers, this requires precise spatial memories and efficient navigation (Collett et al., 2013). Accordingly, eusocial insects are notoriously skilled navigators, learning near optimal routes between their colony nest and specific places based on cues in the local environment and on the panorama (Wehner, 2020). Honey bees and bumblebees can travel several kilometers to visit hundreds of flowers looking for nectar and pollen, and repeat these foraging trips over several days or weeks (von Frisch, 1967). When these resources are patchily distributed in space, foragers tend to develop multi-destination routes (i.e., traplines) by which individuals visit plant patches in stable sequences using the shortest possible path linking all these places (Lihoreau et al., 2012b; Buatois and Lihoreau, 2016). Strikingly however, many non-eusocial insects, including non-central place foraging species, are also excellent at these spatial cognitive tasks. In fact, the seminal experiment demonstrating place learning in insects were conducted in the non-eusocial wasps (*Philanthus triangulum*) in which moving the visual landmarks surrounding the nest of the wasp triggered search around landmarks (Tinbergen, 1932). Fruit flies *D. melanogaster* also develop visual place memories when forced to find a hidden cool tile in a warm arena based on patterns displayed on the panorama (Ofstad et al., 2011). Various butterflies have been observed developing traplines between distant plants (Young and Montgomery, 2020). Dung beetles use environmental cues to navigate in a straight line away from the dung pile as fast as possible (Dacke et al., 2019). Spatial cognition skills supporting efficient navigation are therefore not exclusive to eusocial insects and appear to rely more on ecological factors such as the variability of food sources in time and space rather than sociality *per se*.

3. Concluding remarks

Many cognitive abilities once thought to be required for social interactions and unique to eusocial insects are also being discovered in non-eusocial species (see summary in Table 1). This suggests factors related to foraging ecology, mate selection, reproductive competition, and defense not just sociality may give rise to the rich and elaborated cognitive capabilities found in insects. This also emphasizes the need for further theoretical and experimental research to clarify the relationship between ecology, sociality, and cognition in this group (Lihoreau et al., 2019). We argue this knowledge gap can be filled by overcoming methodological and conceptual challenges.

Foremost among these challenges is a scarcity of model organisms which limits our ability to assess how ecological and life history traits impact cognitive evolution. The large volume of insect behavioral and cognitive research is almost entirely based on highly eusocial ants, bees, and wasps. This is not surprising as these insects are easily maintained in both laboratory and natural environments, display a diversity of cognitive and neuroanatomical traits and have a well-established history of assays used to quantify cognitive

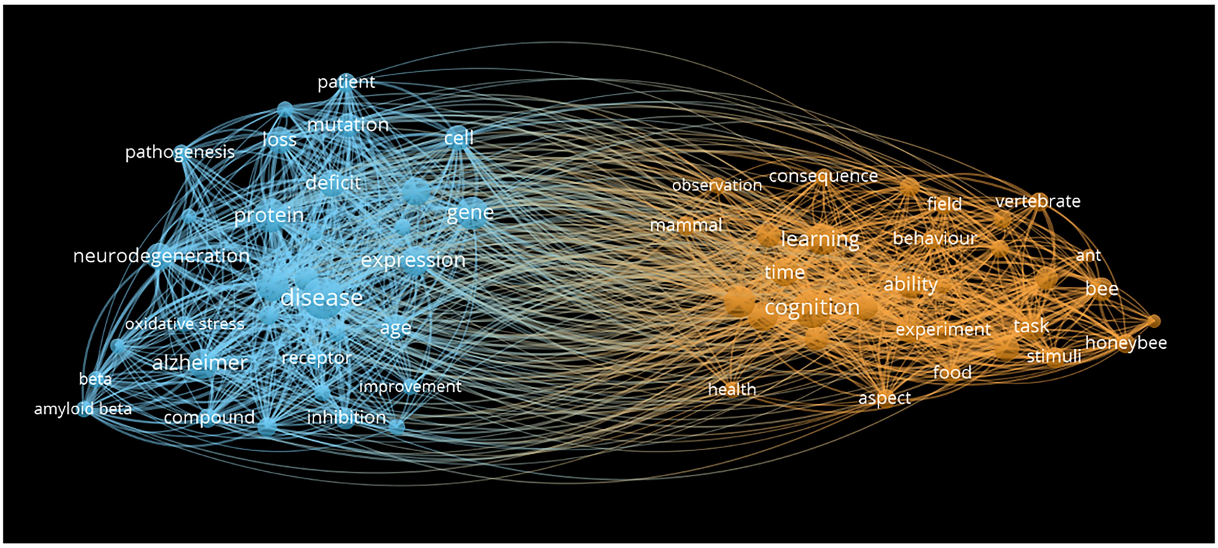

FIGURE 1
Literature search illustrating species biases in insect cognition research. Network map of words extracted from a Web of Science database, downloaded the September 21, 2021 from articles including "insect" and "cognition" either in the title or the abstract. We used VOSwiewer 1.6.16 with binary counting (only the presence or absence of a word counts), including each word that appeared 10 times or more, and removing or replacing irrelevant terms such as plural/singular, homonyms etc (see Supplementary Appendix). In the first cluster (orange), behavioral and learning studies were largely dominated by studies on bees and ants. In the second cluster (blue), molecular biology studies about brain functions were mostly dominated by studies on Drosophila.

variability (Giurfa, 2013). While there have been attempts to compare cognitive abilities across closely related, ecologically distinct, eusocial species (Sheehan and Tibbetts, 2011; Tait et al., 2021), examples are still rare and limited in their power due to the small number of species and phylogenetic groups usually considered in these studies (Harvey and Purvis, 1991). There are, however, other species of eusocial and non-eusocial insects, each with a unique ecology and life history but with very little information regarding their cognitive traits. Comparative analyses investigating such understudied insect clades could go a long way to improving our understanding of the role of ecology and sociality in the evolution of insect cognition, especially given the noticeable taxonomic bias revealed by our review of the literature (Figure 1). The network map of keywords extracted from the Web of Science (titles and abstracts) illustrates research biases for behavioral and learning in bees and ants (orange cluster), in contrast to a functional molecular bias in Drosophila studies (blue cluster).

Another difficulty lies in the unverified assumption that larger behavioral repertoires require larger brains (Godfrey and Gronenberg, 2019). Many fundamental changes in the complexity of a nervous system may not result in measurable volumetric differences, and novel behavior may emerge from minimal rewiring of existing neurons (Chittka and Niven, 2009). Therefore, recent studies point towards a need to consider a new framework for insect comparative cognition, including a comprehensive discussion on their ecology beyond their social status (Lihoreau et al., 2012a; O'Donnell et al., 2015; Farris, 2016; Simons and Tibbetts, 2019). If we want to move from describing cognitive feats in insect models to understand why and how these cognitive abilities have evolved across insects in general, we need to develop more systematic comparative analyses of species with contrasted ecologies (including foraging, mating, and social interactions) and detailed anotomo-functional measures of their brains. It is only by identifying cognitive operations that are specific to social life, the neural circuits they involve, and the ecology and life history of the species, that the social brain hypothesis and its derivatives, as they are currently framed, can be tested and refined.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Human brains *have* shrunk: the questions are *when* and *why*

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Human brain reduction from the Late Pleistocene/Holocene to the modern day is a longstanding anthropological observation documented with numerous lines of independent evidence. In a recent study (DeSilva et al., 2021; *Front. Ecol. Evol.*), we analyzed a large compilation of fossil and recent human crania and determined that this reduction was surprisingly recent, occurring rapidly within the past 5,000 to 3,000 years of human history. We attributed such a change as a consequence of population growth and cooperative intelligence and drew parallels with similar evolutionary trends in eusocial insects, such as ants. In a reply to our study, Villmoare and Grabowski (2022; *Front. Ecol. Evol.*) reassessed our findings using portions of our dataset and were unable to detect any reduction in brain volume during this time frame. In this paper, responding to Villmoare and Grabowski's critique, we reaffirm recent human brain size reduction in the Holocene, and encourage our colleagues to continue to investigate both the timing and causes of brain size reduction in humans in the past 10,000 years.

KEYWORDS

encephalization, *Homo sapiens*, Holocene, Pleistocene, social evolution

Introduction

Our analysis of human brain evolution (DeSilva et al., 2021) was based on robust prior research demonstrating that human brains decreased in volume in the Late Pleistocene or Holocene. This recent reduction has been documented by numerous researchers for nearly 90 years across diverse populations globally (Figure 1; von Bonin, 1934; Weidenreich, 1946; Tobias, 1971; Schwidetzky, 1976; Wiercinski, 1979; Beals et al., 1984; Henneberg, 1988, 1998, 2004; Brown, 1992; Henneberg and Steyn, 1993, 1995; Ruff et al., 1997; Brown and Maeda, 2004; Wu et al., 2007; Bailey and Geary, 2009; Hawks, 2011; Balzeau et al., 2013; Bednarik, 2014; Liu et al., 2014; Stibel, 2021, 2023). The question we asked, then, was not *whether* modern human brain volume was smaller than that of Pleistocene *Homo sapiens*, but *when* this reduction occurred. Addressing this question, we could proceed to infer *why* an organ critical for human survival would decrease in size.

Our original findings that brain size has reduced surprisingly recently (~5,000–3,000 years ago) is consistent with previous research and led to our hypothesis that population growth and knowledge specialization associated with cooperative intelligence led to a decrease in the volume of the brain, which is energetically expensive to develop and operate (Aiello and Wheeler, 1995; Navarrete et al., 2011; Heldstab et al., 2022). We drew parallels with patterns of brain evolution

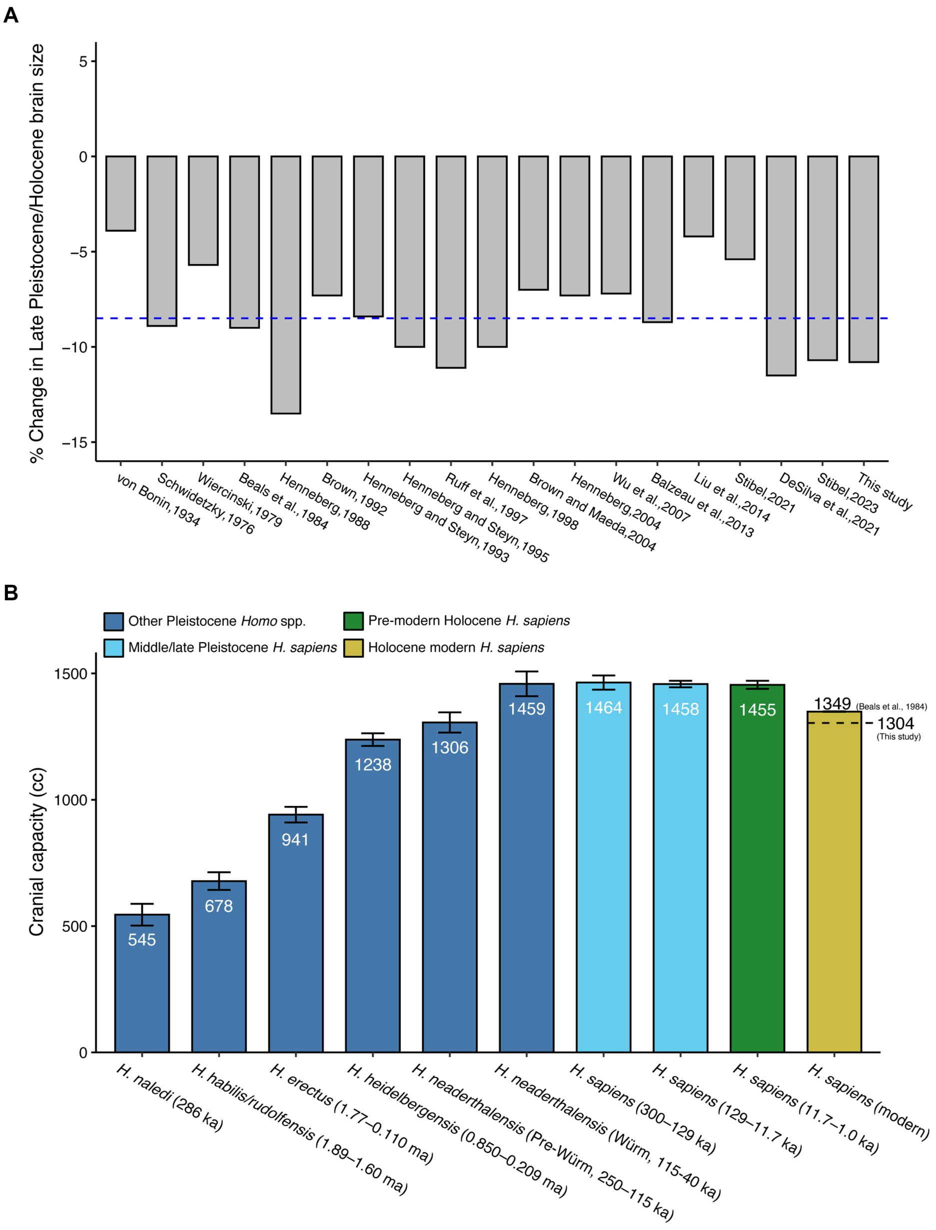

FIGURE 1
(A) Summary of results of published studies in the last *ca.* 90 years reporting a decrease in human brain size in the late Pleistocene/Holocene (N =19). The average reported decrease across all studies is 8.5%, illustrated by the blue dotted line. Percent change was used as published or, if not available in-text, calculated from the average brain volumes published in the study that demarcated the decrease. **(B)** Average brain size (cranial capacity) across members of the genus *Homo* during the Pleistocene and Holocene epochs. Bars are means, with whiskers representing ± one standard error. The last, yellow bar includes both the global mean cranial capacity for modern *H. sapiens* calculated by Beals et al. (1984), and the alternative modern *H. sapiens* mean cranial capacity (dotted line), compiled from recent anatomical and archaeological samples in the current study.

in ants, an entirely eusocial clade in which workers of different species have also undergone selection for both increased and reduced brain size in relation to higher levels of social complexity (Traniello et al., 2022). In ants, the scaling of brain size to body size and brain mosaicism vary with the behavioral and/or cognitive demands of task performance and division of labor, characteristics that are likely to impact brain evolution across diverse taxa, including humans.

Yet Villmoare and Grabowski (2022) recently argued that the dataset from which we based our findings was inadequate for the question being asked. Furthermore, they reassessed our study using portions of our dataset and were unable to detect any reduction in brain volume. Based on their analysis, they conclude that “human brain size has been remarkably stable over the last 300 ka. Thus, hypotheses of recent change are not supported by the evidence.” If these authors are correct, human brain reduction—an established fact for almost a century (Figure 1)—did not occur. In this paper, responding to Villmoare and Grabowski’s critique, we demonstrate that our revised dataset is sufficient for testing trends in brain volume through time and reaffirm recent human brain size reduction.

Recent human brain reduction: what does prior research tell us?

Recent (i.e., Late Pleistocene or Holocene) human brain reduction is not a new idea (Figure 1; Supplementary Table S1) and is not as controversial as Villmoare and Grabowski (2022) suggested. Von Bonin (1934) wrote, "there is a definite indication of a decrease at least in Europe within the last 10,000 or 20,000 years" in the human brain. Noted anthropologists Franz Weidenreich (1946) and Philip Tobias (1971) observed that modern human brain volumes are on average smaller than Pleistocene hominin crania. Schwidetzky (1976) found a decrease in the estimated number of "extraneurons" (following Jerison, 1963) since the Neolithic in parts of Europe. Wiercinski (1979) used linear measurements on 20 different populations in Europe, Africa, Asia, and Australia and reported a reduction in cranial dimensions in 17 of them, concluding that human brain reduction was a post-Aurignacian global phenomenon.

Using a dataset of 5,288 cranial capacities from 122 distinct global populations, Beals et al. (1984) detected a recent decrease in brain size and wrote, "we consider de-encephalization through the last 100,000 years as confirmed." Henneberg (1988) evaluated primarily linear measurements taken on nearly 13,000 skulls and concluded that there had been a 10–17% decrease from the Mesolithic to modern times. Most of these data were obtained on specimens from Europe with additional skulls from northwest Africa and west Asia. Henneberg and Steyn (1993, 1995) identified a similar decrease in brain size in samples from sub-Saharan Africa and Japan. By 2004, Henneberg's global study had exceeded 14,000 samples from 15 thousand years ago (ka) to modern day. He concluded that "Cranial capacity decreased by some 100–150 mL during the Holocene, with most of this decrease occurring during the last 3 Ka." We unfortunately neglected to cite Henneberg (2004) in our original paper and correct the oversight here. We find it compelling that the 3 ka date is consistent with what we found using a different methodology and a different sample (DeSilva et al., 2021).

Using a sample from East Asia and Australia, Brown (1992) reported a recent 10% reduction in cranial capacity. Ruff et al. (1997) used data from Beals et al. (1984) and samples from the Pecos Pueblo (New Mexico, United States) archaeological site and found a Late Pleistocene decrease in brain volume. Brown and Maeda (2004) reported a decrease in the size of the cranium in Chinese skulls from the Neolithic to today—with an accelerating rate of change after 3,500 years before present (BP)—a finding replicated using a different dataset by Liu et al. (2014). Wu et al. (2007) took linear measurements on 718 male skulls from the Holocene of China and reported a 7.2% reduction in calculated cranial volume from the Bronze age to the present. In their study of the Cro-Magnon *H. sapiens* cranium, Balzeau et al. (2013) state that "a decrease in absolute endocranial size since the Upper Pleistocene is noticeable in *H. sapiens*." They based this finding on 15 Pleistocene crania from 25–92 ka and 99 modern human crania from Europe, Africa, Asia, the Pacific islands, and North America. Stibel (2021) found a 5% decrease in brain volume from Pleistocene *H. sapiens* to modern people. In an updated paper, Stibel (2023) reported that brain size in Late Pleistocene (50–12 ka BP) *H. sapiens* was 10.7% larger than in Holocene humans (12 ka BP-present), a statistically significant difference ($p < 0.0001$, t-test).

We recognize that the history of brain science is rife with problematic studies biased by racist and sexist objectives. Furthermore, "brain size" is difficult to objectively measure and different investigators have determined brain mass and/or cranial capacity using distinct methods (see review in Tobias, 1970). Most studies report summary statistics (e.g., Ho et al., 1980) while very few report data from individuals (e.g., Bischoff, 1880). Furthermore, certain regions of the world are overrepresented (e.g., Europe) while there is little data for other human populations. Despite these limitations, independent of measurement technique, brain volume reductions have been consistently reported by researchers for over three-quarters of a century on skulls representing populations globally (Figure 1). It is difficult to accept on scientific grounds that all of these studies are in error.

How big *is* the average human brain?

Villmoare and Grabowski (2022) considered our average reported brain volume for recent modern humans (1,297 cc in DeSilva et al., 2021; 1,304 ± 154 cc in this study) to be lower than other reports showing roughly 1,400 cc, citing Beals et al. (1984), Henneberg (1988), Ruff et al. (1997), and De Sousa and Cunha (2012) as support. However, in the very papers they cite, modern human cranial capacities are less than 1,400 cc on average. Beals et al. (1984) sampled 5,288 crania from 122 different ethnic groups and reported a cranial capacity of 1,349 ± 78 cc. Ruff et al. (1997) supplemented the value reported in Beals et al. (1984) with the Pecos archaeological sample averaging 1,308 ± 123 cc ($N = 29$). Henneberg (1988) used mostly linear measurements to calculate cranial capacities. Where he used directly measured cranial capacities, the weighted average is 1,387 cc ($N = 245$). De Sousa and Cunha (2012) reported an average of 1,392 cc ($N = 551$), though these values are converted from brain weights measured in 20–30 year-olds from Dekaban and Sadowsky (1978). However, the entire Dekaban and Sadowsky (1978) adult dataset ($N = 3{,}399$) indicates an average brain size of 1,334.5 cc ± 205.9. Thus, using identical sources referenced by Villmoare and Grabowski (2022), the range never exceeds 1,400 cc and is instead 1,308–1,392 cc with a weighted average of 1,345 cc ($N = 8{,}961$). Independently, Tobias (1971) reported an identical average of 1,345 cc from "thousands" of measurements.

While it can be problematic to convert brain mass (g) to cranial capacity (cc) (see Tobias, 1970), two equations permit direct comparison. Cranial capacity can be converted from brain weight (g) using Hofman (1983)'s equation:

$$\text{Brain mass}\left(\text{g}\right) = \text{cranial capacity}\left(\text{cc}\right) \times 0.95$$

This equation is derived from brain volume (cc) = cranial capacity (cc) * 0.92 and the specific gravity of human brain tissue = 1.036 g/cm^3. Ruff et al. (1997) established the equation:

$$\text{Brain mass}\left(\text{g}\right) = 1.147 \times \text{cranial capacity}\left(\text{cc}\right)^{0.976}$$

Here, we averaged the results of the two methods which were on average only 1–2% different from one another. For those studies with an equal sex representation, brain size averages between 1,335 ± 206 cc (Dekaban and Sadowsky, 1978; $N = 3{,}399$) and 1,344 ± 137 cc

(Ho et al., 1980; $N = 1{,}261$). Furthermore, Grabowski (2016) reported an average brain mass of 1,299 g (Table 2, p. 180) using data from Bischoff (1880). Converting this value to cc using the equations in Hofman (1983) and Ruff et al. (1997) yields an average of 1,350 cc.

These values, however, almost certainly overestimate the average adult human brain size, given the disproportionate representation of larger-bodied European males in the samples and the known scaling relationship between brain and body size (Ruff et al., 1997; Hawks, 2011; Grabowski, 2016). When smaller-bodied populations from East Asia, sub-Saharan Africa, and Australia are compiled (data from Henneberg and Steyn, 1993; Brown and Maeda, 2004), the weighted sample mean is 1,300 cc ($N = 768$). Therefore, we disagree that our original calculated value of ~1,300 cc for the average human cranial capacity is too low—being slightly larger than Albert Einstein's (~1,291 cc converted from grams; Witelson et al., 1999), and slightly smaller than Walt Whitman's (~1,317 cc converted from grams; Spitzka, 1907). When cranial capacity averages and standard deviations are appropriately weighted by continental populations (Source: https://www.statista.com/statistics/237584/distribution-of-the-world-population-by-continent/), we calculate an average of 1,328 ± 145 cc. Using estimated pre-colonial populations from the year 1,500, we arrive at a weighted average of 1,331 ± 153 cc. Given these data, it is unclear how the commonly reported overestimate of >1,400 cc has entered our collective knowledge.

Critical analysis of human brain volume datasets: statistical approaches

Given the literature cited above, we naturally did not explore *whether* brain volumes had decreased, as that had been clearly established in multiple previous studies, but estimated *when*. To answer this question, we employed a changepoint analysis using the segmented package in R (Muggeo, 2008; details in DeSilva et al., 2021), which led us to compile raw cranial capacities for fossil crania spanning the past 10 million years (Ma), along with a large modern human sample. Compiling these data was not difficult for Miocene and Plio-Pleistocene hominids because endocranial volumes are standard measurements reported for skulls discovered in paleoanthropological or archaeological contexts (e.g. Holloway et al., 2002). Using this dataset, we found statistically significant changes in the rates of hominin endocranial volume change at ~2 [(95% confidence interval (CI): 2.0-2.3) and ~ 1.5 (95% CI: 1.2-1.8)] Ma, findings consistent with previous work on hominin brain size evolution during these periods (Antón et al., 2014; Grabowski, 2016). We disagree with Villmoare and Grabowski (2022) that important crania from diverse taxa such as *Rudapithecus, Australopithecus,* and *Homo erectus*––which we included in our model to contextualize the temporal dynamics of hominin brain evolution before the evolution of modern humans––are not relevant in such discussions (see, for instance, Begun, 2010; Gowlett et al., 2012; Antón et al., 2014; Almécija et al., 2021).

Our use of this particular changepoint analysis was intentional, as it allowed for estimates of breakpoint times and slopes in a large data set that otherwise lacked uniform sampling from each time slice, a widespread issue for most paleoanthropological datasets. Rather, this analysis, implemented by fitting a piecewise linear regression to the data, relies primarily on standard regression assumptions, as pointed out by Villmoare and Grabowski (2022)—i.e., normality and independence of residuals, and homoscedasticity—to generate estimates of slopes and breakpoint locations. Our changepoint approach, while unconventional based on the literature cited in Villmoare and Grabowski (2022), is nevertheless common and consistent with other investigations concerned with estimating the timing of key events in the paleoanthropological record using unbinned, raw time-series data (e.g., Faith et al., 2018; Wynn et al., 2020). For our own analysis, the majority of our time series followed these *a priori* assumptions; in turn, Villmoare and Grabowski (2022) produced estimates for the first two breakpoints in the time series (2.1 and 1.3 Ma) that fell within the 95% CI initially reported in Table 1 of DeSilva et al. (2021).

Yet for recent humans, we were challenged to incorporate sufficient samples to accurately represent modern variation without skewing our data, a constraint we failed to address sufficiently according to Villmoare and Grabowski (2022). They rightly point out that the Holocene portion of our dataset is skewed primarily towards modern humans, an unavoidable taphonomic bias and limitation in our original model that may skew our estimate of when brain reduction occurred towards more recent periods, and, in the worst case, obscure additional, earlier change points. Yet we disagree with their proposed solution: consolidating the individual cranial data into means representing identical temporal slices of 100 years (see Figures 2 and 3 of Villmoare and Grabowski, 2022). Pooling irregularly sampled data into equal-sized time bins runs the risk of diluting trends or introducing spurious ones, depending on data density across time, and particularly on the timing of outlier measurements: e.g., a single outlier data point in a sparsely sampled period would be given the same importance as hundreds of data points from a well-sampled period. Better, less sensitive options include weighted regression models (individual points are assigned importance weights inversely proportional to data density), bootstrapping or resampling (oversampling with replacement from time periods with few data, and/or undersampling without replacement from intervals with high data density), or even log-transforming time measurements (assuming trend direction and changes therein are more important than trend type).

We are not opposed to binning the data to improve *a priori* statistical assumptions (*cf.* Figure 2 of this study), but we also find it problematic to bin data arbitrarily in such a way that is uncritical of the broader question being asked: has there been a significant change in average human brain size since the start of the Holocene? We contend that the reason Villmoare and Grabowski (2022) did not find a Holocene decrease in cranial capacity with their consolidated dataset of means is because the time-averaging process effectively removed the crucial variability in cranial capacity found in the period of interest, i.e., the last 10,000 years. Indeed, when such variability is binned more appropriately (e.g., by geological time periods defined in part by global climate changes) and incorporated into simpler statistical analyses (e.g., *t*-tests; see below analyses with updated data), a strong and significant decrease in modern human brain size across the Holocene boundary is detected (Figure 2), reaffirming our original conclusions (DeSilva et al., 2021).

A related critique by Villmoare and Grabowski (2022) was our use of questionable modern cranial samples from the collection of Samuel Morton at the Penn Museum. We agree this is a problematic dataset because it has been used to promote false and dangerous ideas of white supremacy (Morton Collection Committee, 2021; Mulligan et al.,

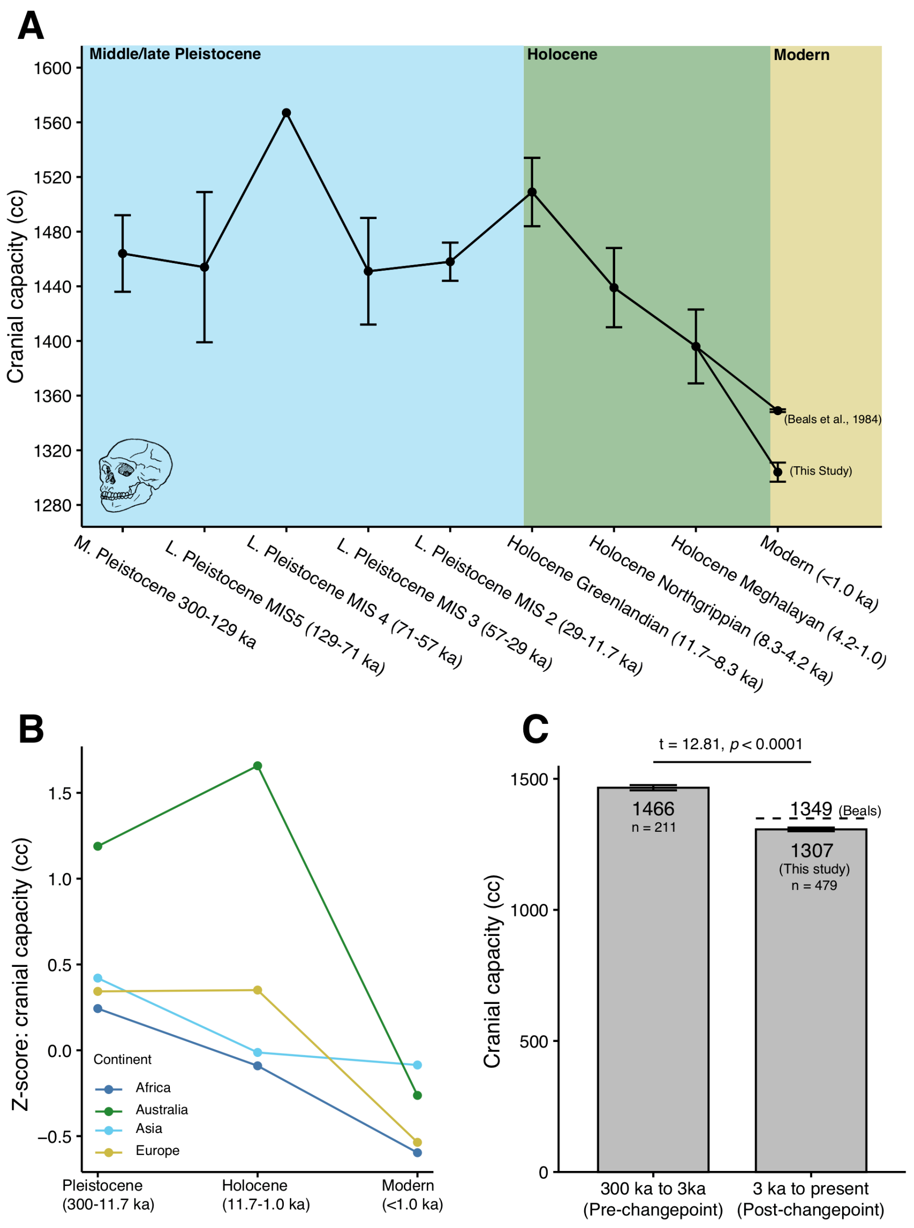

FIGURE 2
Dynamics of brain size reduction (cranial capacity) in *H. sapiens* during the Pleistocene and Holocene. **(A)** Changes in *H. sapiens* cranial capacity over the past 300,000 years, subdivided by geological epochs and climatic milestones, with recent modern samples (<1.0ka) subdivided from the rest of the Holocene. Means represent average cranial capacity, whiskers are ± one standard error. There is only a single cranial capacity reported for MIS 4. **(B)** Changes in the average *H. sapiens* cranial capacity over the past 300,000 years, subdivided by major continental landmasses. Average cranial capacities are presented here as Z-scores (i.e., standard-deviation units). **(C)** Average cranial capacity in *H. sapiens*, before and after the original reduction date of 3,000 years proposed by DeSilva et al. (2021). An average reduction in brain size of 159 cc (using our modern estimate) or 117 cc (using the Beals et al. (1984) modern estimate) after 3,000 years is illustrated. Whiskers are ± one standard error.

2022). Eliminating these data from the present analysis had no appreciable impact on our reported brain volume for modern humans (Supplementary Table S2). In lieu of the Morton data, we have added modern cranial capacity data from the Terry Collection ($N = 94$; VanSickle et al., 2020 via lynncopes.com) and India ($N = 50$; Manjunath, 2002).

Villmoare and Grabowski (2022) also noted that our dataset contained few individuals from a key time-period, 1-5 ka. We added 13 individuals from this time range from Henneberg and Steyn (1993) and Stibel (2021). Additionally, we used the millet seed method to measure cranial capacities of two skulls from the South African archaeological site Byneskranskop (~3.1 ka; Sealy, 2006) and another individual from the older Plattenberg Bay site (~7 ka; Sealy, 2006). We also revised the age of the Pecos Pueblo population to 500 years BP, assuming most of the individuals derive from the Glaze V period. Finally, we removed juvenile Neanderthals ($N = 4$), which were inadvertently included in our dataset, and eliminated one entry of the Liujiang skull, which mistakenly appeared twice. The revised cranial volume catalog is now available as a supplementary Excel file.

Brain reduction in the Holocene

Analysis of our modified dataset shows that Holocene brain reduction remains robust (Supplementary Table S2; Figure 2). On the broadest scale, Pleistocene (300 ka-11.7 ka) *H. sapiens* brains average 1,458 ± 140 cc ($N = 136$). This is effectively identical to the average Neanderthal brain (1,459 ± 182 cc; $N = 14$) from the Würm period (<115 ka; DeSilva, 2018). There is no directional change in brain volume in *H. sapiens* throughout the Pleistocene whether the data are consolidated in consistent time intervals (Villmoare and Grabowski, 2022), or divided by geological stages of the Pleistocene epoch (Supplementary Table S2). This pattern of stasis in *H. sapiens* brain volume changes quite abruptly and obviously in the later part of the Holocene (Supplementary Table S2; Figures 2A,B). We agree with Villmoare and Grabowski (2022) that more samples from this time-period will be valuable for future study. However, we argue that even without any samples from the Holocene, one could identify a change in brain size by simply comparing the chronological "bookends" of the Pleistocene and today. In fact, instead of a change point analysis or data consolidation, a simple *t*-test can effectively evaluate if human brains today differ in volume from humans in the Pleistocene. Using Welch's t-test, the difference between Pleistocene and Holocene human cranial capacities in our dataset is significant ($t = 9.15$, $p < 0.0001$), a result similar to that found in Stibel (2023). Even more granularly, if we were to look at the changes in *H. sapiens* cranial capacity before and after the originally proposed change point (3,000 years) in DeSilva et al. (2021), a *t*-test reveals a significant decrease in human cranial capacity post 3 ka ($t = 12.81$, $p < 0.0001$; Figure 2C).

Because Villmoare and Grabowski (2022) suggest our initial study underestimated modern human brain volumes, we repeated the analysis with brain weight data ($N = 3{,}399$) from Dekaban and Sadowsky (1978)—converted to cranial capacities—and found the same differences ($t = 9.83$, $p < 0.0001$). The Beals et al. (1984) dataset ($N = 5{,}288$), which compiles a larger global sample of cranial capacities and is therefore preferable, also reveals significant differences ($t = 9.04$, $p < 0.0001$, Welch's *t*-test). Therefore, independent of the modern dataset used (e.g., Dekaban and Sadowsky, 1978; Beals et al., 1984; this study), it is clear that there has been, on average, a 100–150 cc reduction in brain volume (Figure 2C). These data are consistent with Henneberg (2004), who similarly found a 100–150 mL reduction in brain volume during the Holocene using measurements on 14,000 crania. These data further mirror a widely recognized Holocene reduction in body size (Ruff et al., 1997; Stibel, 2023) that would be difficult to reconcile with Villmoare and Grabowski's (2022) proposed stasis in brain size.

On finer scales, similar magnitudes of Holocene brain reduction have been documented regionally and across latitudes (e.g., Henneberg and Steyn, 1993; Liu et al., 2014; Stibel, 2023). In other words, human brain volume has decreased by a standard deviation in the last 10,000 years, whether examined locally or globally (Figures 2A,B). It is probable that brain reduction occurred at different rates in different areas during the Holocene—a point also noted in the critique of our initial study. But unlike Villmoare and Grabowski (2022), we view these regional dynamics as integral components of an overarching global reduction in human brain size that defined the last 10,000 years. Holocene brain reduction is not a uniquely human phenomenon; rather, a widespread pattern of brain size reduction is also found in domestic and human-associated mammals during the last 10,000 years—ranging from large hooved taxa like cows, horses, llamas, and pigs to rodents like rats and guinea pigs (Balcarcel et al., 2021a,b, 2022). These findings, combined with our own analyses, speak to the profound effect that the Holocene agricultural revolution and the subsequent rise of complex societies had on the trajectory of human and, more broadly, mammalian brain evolution.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JD, LF, and JT conceived the study and wrote the manuscript. JD, AC, IC, and JS accumulated the hominin brain dataset. JK and JD collected original data in South Africa. LF and II contributed to the statistics. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1191274/full#supplementary-material

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Migrant orangutan males use social learning to adapt to new habitat after dispersal

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Dispersal has been suggested to be challenging, especially for species that heavily rely on social learning for knowledge acquisition. One of the obstacles that migrants face is learning how to cope with an unfamiliar, new habitat, which may involve learning from resident individuals. So far, only very few studies have looked at social learning in migrants after dispersal. Here we examine how migrant male orangutans use a behavior called "peering" (an indicator of observational social learning), to learn from local individuals. In total, we analyzed 4,009 daily dyadic associations with and without peering events of 77 males of the highly sociable Sumatran orangutans (*Pongo abelii*) at the Suaq population and 75 males of the less sociable Bornean orangutans (*Pongo pygmaeus wurmbii*) at the Tuanan population, covering a combined study time of 30 years. Analysis using generalized linear mixed models supported our prediction that migrant males in Suaq preferentially peered at the local adult females. However, in Tuanan, migrants peered mostly at other adult males and local immatures. Migrants' peering rates were highest shortly after their arrival, and significantly decreased with increasing time spent in the area. Migrants in both sites peered significantly more at peering targets' feeding on food items that are rarely eaten within the locals' diet, than at commonly eaten ones and peered significantly more at skill-intense food items than easy-to-process ones. Further, migrants interacted significantly more with the peered-at food item after the peering event, than before, suggesting that they practice the observed behavior. Our results therefore suggest that migrant males use peering to learn new ecological knowledge *after* dispersal (e.g., where and what to feed on), and continue to learn complex skills even *within adulthood*, (e.g., how to feed on skill-intense food items). To do so, migrants selectively attend to the most knowledgeable and/or available individuals, practice the new skill afterwards and even flexibly adjust their learning, e.g., when confronted with intolerant locals or when the need for learning decreases. Together, our study provides important evidence that social learning in great apes expands towards adulthood, an ability which critically impacted also human evolution.

KEYWORDS

observational social learning, peering, eavesdropping, dispersal, migrant male orangutans, skill learning in adults, social tolerance

1 Introduction

Social learning refers to learning that is influenced by the observation of, or by associating and interacting with another individual or its products (Heyes, 1994; Heyes, 2012). Through social learning, individuals can avoid risks and costs of own exploration (Lorenz et al., 2011), acquire knowledge faster (Custance et al., 2002; van Schaik and Burkart, 2011) and benefit from the "wisdom of the crowd" (Toyokawa et al., 2019). Accordingly, social learning is a well-documented learning mode in a variety of taxa, ranging from invertebrate (Grüter and Leadbeater, 2014) to vertebrate species (Ferrari et al., 2007; Wilkinson et al., 2010; Aplin et al., 2015; Sasaki and Biro, 2017) and is especially prevalent in humans and non-human primate species (Whiten et al., 1999; van Schaik et al., 2003; Gariépy et al., 2014). Social learning is also the prerequisite for the emergence of traditions and cultures (Whiten et al., 1999; van Schaik, 2010).

Wild great apes are known to heavily rely on social learning for skill acquisition and live highly cultural lives, with chimpanzees and orangutans known to have the most sophisticated and diverse non-human cultures in nature (Whiten et al., 1999; van Schaik et al., 2003). Extensive research on wild primates suggests that the social learning strategies during lifetime development follow a three-phase model: in the first phase, infants benefit from extensive vertical learning from the primary care giver, usually their own mother (Lehmann et al., 2013; Whiten and van de Waal, 2018). This is then followed by a second phase, where juveniles gradually learn obliquely from a wider array of group members with increasing age (Whiten and van de Waal, 2018; Ehmann et al., 2021). A third phase of potential horizontal social learning would take place when individuals reach sexual maturity and disperse to new areas, where they encounter new and unfamiliar ecological and social circumstances (see below). Mathematical models support these shifts in role model choice (who to learn from) and they suggest that during these three phases also the context and content of social learning flexibly shifts, according to the requirements of the respective life stage the individual is situated in (Lehmann et al., 2013).

The context of social learning greatly depends on the knowledge, or the deficit thereof, of the learner which can vary considerably depending on an individual's age and experience. Potential social learning contexts are foraging, mating, or migration (Brown and Laland, 2003). One pathway during context learning is to adopt a behavior of a conspecific, when e.g. being uncertain or when the own established behavior is unproductive (Laland, 2004), with an information flow going from an expert to the naïve or not knowledgeable individual, independent of learners' age classes (Henrich and McElreath, 2003). In terms of the content of social learning, skills such as "knowing how" are dominantly shared between parents and offspring, whereas information about the current state of the environment or conspecifics ("knowing where", or "knowing who"), as well declarative knowledge ("knowing that", van Schaik, 2010) is shared among adults. However, it needs to be kept in mind that individuals must balance the costs and benefits of social learning. Costs can include attending to outdated information or when competing over resources with non-related conspecifics, which are reluctant to share information (Emery and Clayton, 2001; Clayton et al., 2007; Bugnyar et al., 2016).

Through these shifts in role model selection, context and contents of social learning, individuals can build up and refine their repertoires on which they will rely during adulthood (Lehmann et al., 2013). This implies that by the time individuals reach adulthood, all necessary skills and knowledge are present, representing accumulated knowledge between and within generations (Schuppli and van Schaik, 2019). It also implies that most social learners are young, and that the frequency of social learning decreases significantly with increasing age (Schuppli et al., 2016c). However, these theoretical models only apply, when the ontogeny follows a linear development within a stable environment and they do not consider the occurrence of dramatic changes in social and environmental conditions. Natal dispersal, i.e. when individuals permanently depart from their natal area, might be a disruptive phase, where individuals cannot rely on information already collected, but must – in extreme cases – start all over, learning about a new ecological and social niche. Social learning may be one way of coping with such sudden changes (Whiten, 2017; Gruber et al., 2019; Whiten, 2021). So far it has not been systematically studied how adult migrant primates make use of observational forms of social learning, to potentially expand and update their repertoire after dispersal for optimal resource exploitation.

Many primate species show sex-biased natal dispersal, which takes place when individuals reach sexual maturity (Cheney and Seyfarth, 1983; van Noordwijk and van Schaik, 1985; Suzuki et al., 1998; Pusey, 2004). The evolution of sex-biased dispersal is a result of the species' experienced trade-off between the costs of staying e.g. risk of inbreeding and resource competition and the benefits of staying in known areas with supportive relatives (Pusey and Packer, 1987; Silk and Brown, 2004; Widdig et al., 2017). Dispersing individuals, on the other hand, face energetic, time, and opportunity costs (Ferreras et al., 2004; Bonte et al., 2012); as well as an increased risk of mortality due to predation (Cheney and Seyfarth, 1983) and starvation (Dittus, 1977). Individuals that leave their natal area when reaching sexual maturity and disperse, might experience high levels of aggression from hostile residents after arrival, whether the species exhibits male or female dispersal (van Noordwijk and van Schaik, 1985; Suzuki et al., 1998; Kahlenberg et al., 2008). In addition, migrants may lack relevant ecological knowledge in their new habitat, which is likely to be especially true for long distance dispersers (Isbell and Vuren, 1996).

Systematic research on how social learning is used to cope with challenges of dispersal under natural conditions and how learning changes over extended periods of time is so far missing. However, experimental studies in wild vervet monkeys (*Chlorocebus pygerythrus*) found that migrants selectively learn from philopatric females about how to open an "artificial fruit" (van de Waal et al., 2010) or a foraging box (Bono et al., 2018) and would even abandon learned food preferences in favor of the local norm (van de Waal et al., 2013). Luncz and Boesch (2014) reported that one migrant female chimpanzee (*Pan troglodytes)* progressively changed her own socially learned nut-cracking technique after immigrating to a new group where individuals show a different technique. Other studies also reported how migrants would adapt in different aspects of the social domain in different primates (*Pan troglodytes*, Nakamura and Uehara, 2004; *Papio anubis*, Sapolsky and Share, 2004). Furthermore, there is evidence for social learning in the social context in that migrants and locals adapt to strong intergroup aggression in chimpanzees (Wrangham and Glowacki, 2012; Wilson et al., 2014) or to highly affiliative behaviors in bonobos (*Pan paniscus*, Sakamaki et al., 2015; Fruth and Hohmann, 2018). However, unlike in orangutans (see below), these species live in social groups where migrants are expected to experience a certain degree of conformity in the form of peer or group pressure, which likely influences the migrants' social learning to manage social relationships, maybe in addition to learning the local ecology.

Orangutans are especially suited to investigate social learning because of their slow development (Wich et al., 2004; van Noordwijk et al., 2018) which provides growing individuals with plenty of opportunities of social learning from mothers and others (Schuppli et al., 2016a; Ehmann et al., 2021). Wild orangutans have been shown to be highly neophobic (Forss et al., 2017), likely to avoid risks and costs of individual exploration (Forss et al., 2015). Despite their strong novelty avoidance, wild orangutans live in skill-intense feeding niches and rely on broad and difficult-to-acquire skill repertoires (Marshall et al., 2009; Schuppli et al., 2016b). Immature orangutans have to learn more than 200 different food items and skill intense foraging techniques, a process which takes around 12 years (Jaeggi et al., 2010; Schuppli et al., 2016a), while items and techniques that are rare or difficult require the highest learning effort (Schuppli et al., 2016c). During this time, immature orangutans also gain increasing independence from their mothers as evident in a decrease in time spent in physical contact, as well as behavioral changes (van Noordwijk and van Schaik, 2005; van Noordwijk et al., 2009; Mendonça et al., 2017). Results by Schuppli et al. (2016a) suggest that virtually all learning in immature orangutans happens *via* observational social learning in the form of peering, first from their mothers and with increasing age from other individuals (Schuppli et al., 2016c; Schuppli and van Schaik, 2019). In addition, with increasing independence from the mother, immature orangutans show sex-specific interest in their peering behavior towards specific classes of adult role models, presumably in preparation for their later dispersal patterns and adult role (Ehmann et al., 2021).

Peering is the attentive close range watching of an individual (hereafter "peering target") with such avid attention and physical closeness, that it allows the peerer to witness the details of the peering targets activity (Figure 1, see full definition Table 1 in methods). Peering has been established as a measure of social learning in the wild: e.g. immature chimpanzees (Matsuzawa et al., 2001; Lonsdorf et al., 2004), immature capuchin monkeys (*Cebus apella*, Ottoni et al., 2005) adult bonobos (Idani, 1995; Péter et al., 2022), and in Sumatran (*Pongo abelii*) and Bornean orangutan immatures (*Pongo pygmaeus wurmbii*, Schuppli et al., 2016a;

FIGURE 1
Migrant orangutan male (on the right side) peering at an adult local female (on the left side), feeding on termites within a dead branch, species *Pongo abelii*. Photo courtesy SUAQ Project, www.suaq.org.

TABLE 1 Definitions of the focal behaviors used as a measure of observational social learning and practice behavior, changed after Schuppli et al. (2016c).

Behavior	Definition
Peering	The peerer is directly looking at the action of another individual (peering target), sustained over at least 5 seconds, and at close enough range that enables the peering individual to observe the details of the action (within 2 meters in the feeding- and within 5 meters in the nest-building context). The peering individual faces the peering target and shows signs of following the actions of the peering target by head movements, which indicates attentive interest in the action of the target.
Peering-practice-behavior	The peerer interacts with the peered-at item before or after a peering event, which can include the manipulation, handling and feeding on the peered-at item.
Interaction rate	Interaction rate is defined as the number of interactions the peerer has with the peered-at item, controlled for the time the peerer spent in a given food patch in which the peering occurred (i.e., feeding locations in trees of the same species that are less than 10 meters apart). The interaction rate is categorized as "before" (time between patch entry and the peering event) and "after" the peering event (time between peering event and patch exit).

Ehmann et al., 2021), as well as in captivity: e.g. in adult Sumatran and Bornean orangutans (Stoinski and Whiten, 2003; Dindo et al., 2011), adult bonobos (Stevens et al., 2005) and adult chimpanzees (Yamanashi et al., 2020).

Sumatran orangutans live in individual based fission fusion systems (van Schaik, 1999), but are highly sociable, whereas Bornean orangutans are less sociable, spending most of their time alone (van Noordwijk et al., 2012). The differences in sociability are linked to differences in food availability on the two islands. Sumatra tends to have higher forest productivity (Wich et al., 2011), which has likely led to the higher evolved levels of sociability and tolerance due to reduced competition over resources (Knott et al., 2008; Knott et al., 2010; Schuppli et al., 2017). However, in both species males disperse at the onset of reaching maturity, while females are the philopatric sex and settle close to their natal area (Arora et al., 2012; Nietlisbach et al., 2012; van Noordwijk et al., 2012). By the time males disperse for the first time, they are still in a state named "unflanged" which resemble the female appearance (Morrogh-Bernard et al., 2011). Most males later go through a secondary growth spurt and develop into so called "flanged" males with secondary sexual characteristics, including cheek pads (Utami Atmoko et al., 2002; Dunkel et al., 2013). Flanged and unflanged males may eventually settle and become residents, or remain transient and move between areas (Delgado and van Schaik, 2000). Although it is not exactly known where they disperse to, studies showed that males travel over long distances and even cross physical barriers such as rivers and mountains (Nietlisbach et al., 2012; Nater et al., 2013). Additionally, males are known to have large overlapping home ranges (van Schaik, 1999; Singleton and van Schaik, 2001). Due to their long-distance dispersal, migrants are expected to possess different knowledge compared to the local resident females (Schuppli and van Schaik, 2019). Since differences in socially learned skills are even visible between individuals living in the same study area (Jaeggi et al., 2010) and in close communities with similar habitats (Bastian et al., 2010), males that have recently arrived are expected to lack knowledge on the local area, in contrast to males who have already spent more time in the area or resident females. Accordingly, (Schuppli and van Schaik, 2019) showed that local adult females hardly peer at all.

The aim of this study is to investigate whether migrant male orangutans use peering as means to socially learn about the local ecology of their new habitat after dispersal. To do so, we investigate the peering content, peering target choice, and effects of arrival time on males' peering frequencies. Specifically, we will test the following predictions:

1. Peering in the feeding context: If males peer to learn about the local ecology then they should mostly peer at orangutans feeding on food items which are rare, because those items are less likely to be known to the males. We do not expect an effect of the processing intensity (i.e., complexity) of the food item on peering rates, because adult migrant males should be familiar with many different processing techniques, including complex ones (prediction 1a). Furthermore, we predict a significant increase in practice rates of the peered-at behavior after a peering event (prediction 1b).
2. Peering target choice: If peering is used to learn about the local ecology, then it should be directed at role models which are most knowledgeable of the local ecology. Female orangutans spend their lives in their natal area, therefore females likely have the highest knowledge of their home range. Consequently, we predict that males peer at adult females more often than at any other age-sex class (prediction 2).
3. Peering over time: With increasing time spent in the area, males should gain more knowledge about its ecology and thus, the need to learn should decrease. Accordingly, we predict that peering rates will decrease with increasing time spent in the study area (prediction 3).

2 Methods

2.1 Data collection and study animals

The data for this study were collected from 2003 to 2018 on the population of Bornean orangutans (*Pongo pygmaeus wurmbii*) at the Tuanan research station, in Central Kalimantan, Borneo, Indonesia, and from 2007 to 2020 on the Sumatran Orangutan population (*Pongo abelii*) at the Suaq Balimbing Research Station, in South Aceh, Sumatra, Indonesia. At both sites, data were collected during nest to nest follows of focal individuals, following a standardized behavioral observation protocol and using instantaneous scan sampling at two-minute intervals (see protocol here http://www.aim.uzh.ch/de/orangutannetwork.html). A total of 157 observers contributed to data collection. All of these observers had passed through an extensive training period and achieved at least an 85% level of agreement with experienced observers. As part of the scan sampling, the activity of the focal animal as well as the distances to all other individuals in association with the focal individual (see below) were noted at two-minute intervals. Whenever the focal individual was feeding, the details were noted (see below). Additionally, behaviors of special interest to the objectives of the ongoing research at the sites, including all social interactions, were recorded at an all occurrence basis and described in detail. At both sites, all occurrence data on peering behavior (see Table 1 for definitions) were collected. For each peering event, the identities of the peerer and peering target as well as the duration of the peering event were noted. Whenever possible, the reactions of the peerer and the peering target were described in detail. This included who approached to peering distance, as well as who ended the peering event and how this was achieved (see Figures S4–S6 in Supplement A). The peered-at activities of the peering targets were divided into four categories: social, nesting, feeding and other behaviors (i.e. included activities like moving, resting, defecating, etc.). Following (Schuppli et al., 2016c), we defined nest peering distance as ranging from 0 to 5 meters between the peerer and the peering target(s) whereas for all other contexts (including feeding context) peering distance was defined as ranging from 0 to 2 meters.

For peering in the feeding context, the species of the consumed food item were noted, as well as the parts eaten by the individual (i.e., leaves, flowers, fruits, bark, pith, vegetative matter, but also insects and their products). Throughout this study, we refer to the combination of species name and the part eaten, as a “food item” (Bastian et al., 2010). Food items that were not recognized during a follow were photographed for later identification, consulting experienced staff members or the project’s extensive botanical record. In total, we had ~28,000 hours of feeding data, and a total of ~42,000 male focal follow hours available (Supplement A, Table S1). The feeding data was used to create frequency scores for food item combinations for both sites separately, by ranking each food item combination according to the time it had been eaten by the local females and their independent offspring. The frequency of particular food items being eaten varied greatly; from 2 minutes to 2500 hours throughout the record of feeding events in the populations. To control for the influence of temporal variation in food availability on social learning at both sites, we used the fruit availability index (FAI), calculated each month as the percentage of fruiting trees in established phenology plots (Marshall et al., 2009; Vogel et al., 2015).

We defined a daily dyadic association as a focal male spending time with another individual of any age-sex class, within 50 meters. Every encountered orangutan, either as an association partner of the focal (henceforth called “association member”) during a focal follow or during opportunistic encounters, was identified and recorded on a daily basis. We distinguished between four categories of association members: adult females, immatures, unflanged males and flanged males. All adult male orangutans, including 88 unflanged and 64 flanged males, were classified as adult migrants due to the species’ male-based dispersal. For each male, we estimated their arrival date in the study area based on the date he was first encountered by the research teams. We also computed a continuous record of the number of months they were encountered in the study area which represents the absolute minimum number of months they had spent in the study area (see Figure S3 in Supplement A). As locals we considered all adult females with their immature offspring, who had not reached sexual maturity (usually at 12–15 years of age, van Noordwijk et al., 2018). In total, we included 49 adult females and their 80 immature offspring from both sites into the analysis as association members. Since no peering was directed at flanged males, we excluded them as peering targets from the analysis.

In total, we compiled 4009 daily dyadic associations, with a total of 207 daily dyadic associations with 534 peering events and 3919 daily dyadic associations without peering events, of a total of 1350 dyads from both sites (see data overview in Supplement A, Table S1). To account for different durations of peering events, they were weighted according to their duration, e.g., a peering event with a duration of minimum of 5 to 120 seconds got a peer count of 1, from 120 to 240 seconds got a peer count of 2 and so on (Supplement A, Table S2). Since data availability differed for each prediction tested, the sample size varies across the different models (see Supplement A, Table S3) for amount of data used per model.

2.2 Data analysis, data sets and model structures

2.2.1 Peering in the feeding context

To test the effects of food item frequency and processing intensity on peering (prediction 1a), we calculated the number of peering events the migrants directed at peering targets feeding on different food items. To assess the level of manual and oral processing steps needed to consume each item (i.e. food item processing intensity), each food item was classified by the number of steps needed to process it before ingestion. Processing steps range from 0 (e.g., pick and eat a leaf) up to 5, which represent the most sophisticated form of food processing skills including tool use, which is regularly seen only in the Suaq population (Meulman and van Schaik, 2013; Schuppli et al., 2016c, see Supplement A, Table S4). This analysis explored patterns within male peering behavior and thus only considered days where actual peering occurred. To control for varying opportunities to peer at different food items, we included the time each food item was eaten by the peering targets while being in association with the peerer. In total, we analyzed 789 daily dyadic events with a migrant peerer associating with another orangutan of any age-sex class in 74 dyads. The structure of model 1a included the males’ peering count as response variable and the time the peering target had spent feeding on the respective food item per day as an offset term. Food item frequency and processing intensity were included as predictors and FAI as control variable. Additionally, we included date, food items and dyad as random effects and random slopes of dyad over FAI and processing intensity into the model.

To test if males practice what they peered at (prediction 1b), controlled for the time the peerer spent in a given food patch with a peering target, we calculated the migrants' interaction rate with the peered-at item, before and after the peering event (see Table 1. for definitions). For this question only data from the Suaq population were available, since patch entry and exit times were not systematically recorded in Tuanan. In total, we here analyzed 126 dyadic events with a migrant peerer associating with another orangutan of any age-sex class of 15 different dyads, including 12 migrant males. Model 1b included the males' number of interactions with the food item before and after peering as response variable, and the time the peerer spent in the given food patch per dyad as an offset term. We included the condition (before and after the peering event) as a categorical predictor and FAI as control variable. Additionally, we controlled for the dyad and date by including them as random effects.

2.2.2 Role model choice

To test if males learn about the local ecology by peering most at local philopatric females, who are expected to possess the most knowledge on the area (prediction 2), we used the full record of dyadic associations between migrants and individuals of the different age-sex classes during our study period. For each male, we calculated the number of peering events directed at each dyadic association partner, controlled for the time they had spent in association (i.e., between 0 and 50 meters) during a particular three-month period (hereafter "quarterly peering count"). This included all daily dyadic associations migrants spent in association with orangutans of the different age-sex classes, i.e. days with both peering absent and present. We here used 2,426 daily dyadic events with a migrant peerer associating with other party members of any age-sex class (1350 dyads). Model 2 included the males' quarterly peering count as response variable and the time in association with the dyad partner per day as an offset term. In this model, we included an interaction between the peering targets' age-sex class and the site due to the expected differences in social tolerance of individuals of different age-sex classes. Additionally, we controlled for male ID, dyad and year by entering them as random effects into the model.

2.2.3 Peering over time

To test if male peering decreases with increasing time spent in the area due to their increase in local knowledge (prediction 3), we included the males' quarterly peering count as response variable and the time in association with the dyad partner per day as an offset term. We then linked this data with the number of months the migrant peerer had been recorded to be present in the area, and controlled for the respective FAI value at the specific association date. Since in 3,919 out of all 4,009 total dyadic observations (97%, Supplement A, Figure S1) the males' peering count was zero, we here present a model excluding all dyadic associations without peering. Therefore, we only explored patterns within male peering in this analysis. Model 3 included the time a migrant had spent in the area (in months) as a continuous predictor and the FAI as continuous control variable. We further controlled for the site, dyad, year and ID by including them as random effects. Additionally, we included random slopes of ID, dyad and year over FAI and the continuous predictor present month in area. In total, we analyzed 149 daily dyadic events with a migrant peerer associating and peering at another party member of the different age-sex classes, summed up quarterly.

2.3 Statistical analyses

All analyses and graphs were done in R, version 4.2.0 (R Core Team, 2023). We used generalized linear mixed models (GLMMs) with a negative binomial family distribution (model 1a, 2) or a Poisson family distribution (model 1b, 3) as implemented in the glmmTMB package (Brooks et al., 2017). Throughout all analyses, the fruit availability index (FAI), food items frequency and processing intensity were z-standardized across sites. We ran full-null model comparison using likelihood ratio tests (LRT) by using the "anova" function, with the null model containing the random effects and the control variables only (Fox and Weisberg, 2019). If the comparison revealed that the full model fitted the data significantly better than the null model, we assessed the effect of each predictor in the full model using the "drop1" function of VGAM package (Yee, 2020). In the case that the GLMM included categorical predictors, *post-hoc* tests were done using Tukey pairwise comparisons implemented in the emmeans package (Searle et al., 1980). Furthermore, all models were tested for overdispersion (Mundry, 2022) and zero-inflation using the DHARMa package (Hartig, 2022). Random slopes were included in model 1a) and 3) and excluded from all other models to achieve model convergence, see Supplement B for random effects and random slopes output for each model. The dispersion parameter of all models ranged from 0.56 to 1.11, the zero-inflation ratio of observed to predicted zeros from 0.98 to 1.07 suggesting no critical cases. We assessed the overall fit of the models by calculating the conditional pseudo delta R^2 using the MuMln package (Nakagawa et al., 2017; Bartoń, 2023). We validated the models' stability using influence diagnostics of the "glmmTMB_stability" function following (Mundry, 2022), which compares model estimates from the full dataset with those that were extracted from the data excluding cases (individuals and dyads) one at a time. We detected no influential cases, since the direction of the effects were consistent in all models (Supplement B, detailed model output). Additionally, all models were checked visually to assess whether they fulfil the model assumptions (Harrell, 2015). Using the vif() function from the car package (Fox and Weisberg, 2019), we further detected no effect of multicollinearity among the fixed effects in any model, with the variance inflation factors ranging from 1.00 to 2.39. Alpha level throughout all tests was set on p = 0.05 with significant values marked with bold fond. Trends were interpreted for p values ranging between > 0.05 and 0.08 marked with bold fond and a dot.

3 Results

Descriptively, we found that in absolute numbers, most (67.84%) of the peering events by male migrants happened in the feeding context (Supplement A, Figure S2; Table S6). When analyzing the males' peering behavior in the feeding context in more detail, we found several lines of evidence that migrant orangutan males use peering to learn about local food items (prediction 1a).

1a Peering in the feeding context: food item frequency and processing intensity

The full-null model comparison revealed that the set of predictor variables used had a significant influence on the migrants' peering rates (full-null model comparison, LRT: $X^2 = 19.62$, $df = 4$, $P < 0.001$). Specifically, the full model revealed that the food item processing intensity had a significant positive effect on migrants' peering rates, with migrants peering significantly more at skill-intense food items, than at easy-to-process ones (Table 2; Figure 2). Furthermore, food item frequency had a significant negative effect on peering rates, i.e., migrants peered significantly more at rare food items than at common ones (Table 2; Figure 3).

1b Peering practice behavior

The full-null model comparison revealed that the predictor variable used had a significant influence on the migrants' interaction rate with the peered-at item (full-null model comparison, LRT: $X^2 = 951.403$, $df = 1$, $P < 0.001$). The full model showed that migrants interact significantly more with the peered-at item after the peering event than before the peering event (Table 3; Figure 4).

2 Role model choice

The full-null model comparison revealed that the predictor variable used had a significant influence on the migrants' peering rates (full-null model comparison, LRT: $X^2 = 49.57$, $df = 5$, $P < 0.001$). The full model revealed a significant interaction between the age-sex class of the role model and study site (LRT: $X^2 = 15.02$, $df = 2$, $P < 0.001$, Table 4; Figure 5). The interaction showed that migrants of the more sociable population Suaq peered significantly more at adult females and local immatures, compared to Tuanan, the less sociable population. Specifically, the *post-hoc* test showed that migrants in Suaq peered most frequently at local adult females, followed by unflanged males and local immatures (Supplement A, Table S5; Figure 5). In contrast, migrants in Tuanan peered most often at unflanged males, followed by local immatures and the least at local adult females (Supplement A, Table S5; Figure 5). At both sites, no peering was directed at flanged males.

3 Peering over time

The full-null model comparison revealed that the predictor variable used had a significant influence on the migrants' peering rates (full-null model comparison, LRT: $X^2 = 12.94$, $df = 2$, $P < 0.001$). In more detail, migrants' peering rates decreased significantly with increasing time spent in the area (Table 5; Figure 6).

4 Discussion

Our study investigated whether migrant male orangutans use peering behavior to socially learn about the food items in their new habitat after dispersal. Therefore, we examined the content of migrants' peering behavior, their role model choice and subsequent practice of the learned behavior in two different orangutan populations using data collected over 30 study years. Further, we analyzed how the migrants' peering rates would change with increasing time spent in the area. We found several lines of evidence that male migrant orangutans indeed use peering behavior as an observational form of social learning and utilize it in a flexible and selective manner. The results suggest that migrant males use peering to acquire new ecological information after dispersal, including where and what to feed on (on rare and common items) and continue to learn complex skills even within adulthood (like how to feed on skill-intense food items). To do so, migrants selectively attend to the most knowledgeable and/or

TABLE 2 The effect of the food items' frequency and processing intensity on the migrants' peering behavior analyzed using a GLMM with a negative binomial family distribution.

Factor	Factor type	Estimate	SE	Lower CI	Upper CI	χ^2	*df*	*P*
Intercept	Intercept	−0.04	0.2	−0.5	0.31	–	–	0.85
Frequency	**Predictor**	−0.38	0.19	−0.76	−0.04	3.521	1	**0.047**
Proc. Intensity	**Predictor**	0.38	0.16	0.15	0.6	10.368	1	**< 0.001**
FAI	Control	0.13	0.2	−0.29	0.51	0.063	1	0.518
Site (Tuanan)	Control	−0.72	0.35	−1.53	−0.06	3.574	1	**0.043**

Shown are the model estimates, with standard errors (SE), lower and upper confidence intervals (CI), Chi-square of predictors (χ^2) and degrees of freedom (df). Analysis is based on N = 789 daily dyadic observations of migrants peering at peering targets feeding on different food items, on days where peering occurred. The conditional pseudo delta R^2 for this model was 0.36.

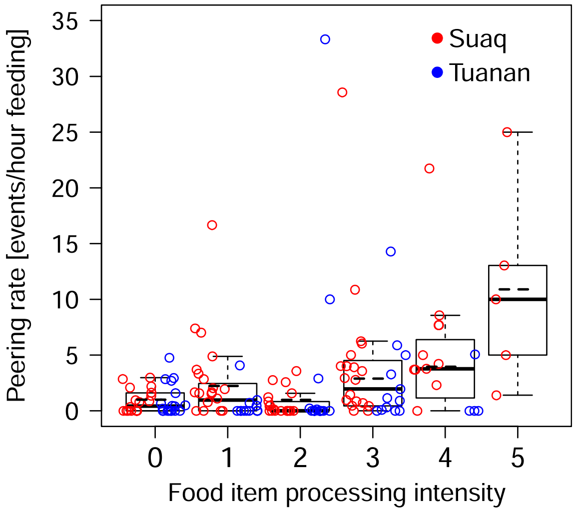

FIGURE 2
Migrants peering rates at food items with increasing food item processing intensity, ranging from 0 of being very easy to process, to 5 of being very skill-intense to process, controlled for the time in hours the peering target fed on the item. Each dot represents the average rate per individual male per complexity level and food item combination. The mean peering rate over all males is shown as a dotted line, the median is indicated by the horizontal line, the upper and lower quartile are depicted by the box and the minimum and maximum values are shown by the whiskers.

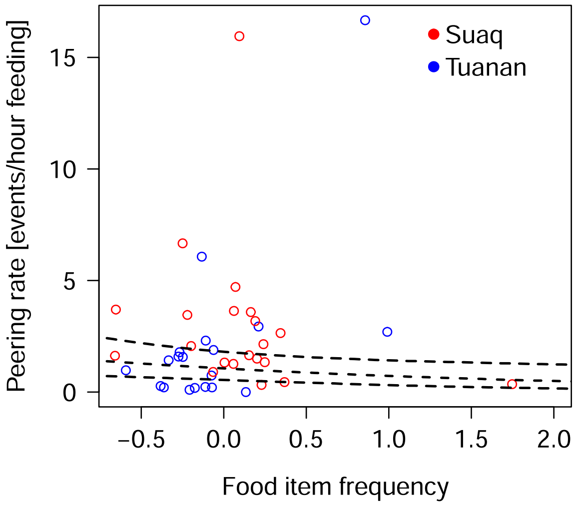

FIGURE 3
Migrants peering rates at different food items, depending on the items frequency in the local females' and their independent offspring's diet, ranging from being very rare (−0.5) to very common (1.0) controlled for the time the peering target fed on the respective food item. Each dot represents the average rate per individual male per food item combination. The dashed line depicts the fitted model, with its confidence limits for the predictors as dotted lines.

available individuals of the study areas, subsequently practice the new skill, and flexibly adjust their learning, e.g. when confronted with intolerant locals or when the need for learning decreases. Crucially, we found these effects in two orangutan species which show differences in their levels of sociability and tolerance originating from differences in food availability between Borneo and Sumatra (Wich et al., 2011).

4.1 Peering contexts and food peering

In line with our predictions we found several lines of evidence that migrant orangutan males use peering to learn about local food items (prediction 1a). Descriptively, we found that the majority of the migrants peering events happened in the feeding domain. First, and against our initial prediction, we found that migrants' peering increases with increasing processing intensity of a food item. The highest levels of peering were shown at peering targets engaging in the most skill-intense forms of food processing skills, including tool use. It is generally assumed that in non-human primates skill learning (knowing how) is completed by the end of the developmental period (van Schaik, 2010; Schuppli et al., 2016a). In immature orangutans, adult levels of easy feeding techniques are achieved shortly after weaning, whereas the more skill intense techniques of food consumption need more time, but seem to be mostly in place before reaching adulthood (i.e., age at first reproduction) and thus before dispersal (Schuppli et al., 2016a). Furthermore, findings on humans suggest that adults are able to transfer existing skill and knowledge to solve new problems (Nisbett, 2009; van Schaik and Burkart, 2011). Our results however indicate that orangutan male migrants are lacking knowledge on local food items in the study areas and use peering to learn how to consume the easy-to-process, but especially the skill-intense local foods.

Second, we found that migrants' peering significantly increases with increasing rarity of the respective food item in the locals' overall diet. This result is in line with our prediction, that migrants use peering to learn what they can eat in their new habitat, as well as where and when to find it. Similar findings have been reported in immature orangutans (Schuppli et al., 2016c) and in wild capuchin monkeys, were individuals were found to pay more attention to foods, that are rare in their diet and that are difficult to process (Perry and Jimenez, 2006). It is known that with increasing geographic distance the similarities in habitat ecology, composition and culture decreases, which increases the likelihood that the migrants which are known to disperse over long distances might not know especially the rare local food items (van Schaik et al., 2003; Russon et al., 2009; Krützen et al., 2011). However, it is not known, if items that are commonly eaten at Suaq and Tuanan, are commonly available items in other areas. Furthermore, food species might be commonly eaten locally but still rarely eaten in other areas, due to diet preferences (Bastian et al., 2010). Therefore, to fully test our prediction we would have to gain knowledge about food item availability in the males' natal populations.

Third, we found evidence that migrant males' practice what they have learned, e.g. by interacting more frequently with the peered-at item after the peering event than before (prediction 1b). Similar selective explorative behavior has been studied in immature orangutans suggesting that peering was followed by increased rates of practice behaviors with the peered-at items (Schuppli et al.,

TABLE 3 Results of the GLMM with a Poisson family distribution of the migrants' interaction rate with the food item before and after the peering event (condition) for the *Suaq population only*.

Factor	Factor type	Estimate	SE	Lower CI	Upper CI	χ^2	*df*	*P*
Intercept	Intercept	−20.18	14.63	−56.51	2.59	–	–	0.168
Condition (Before)	**Predictor**	−4.39	0.11	7.34	7.79	951.4	1	< **0.001**
FAI	Control	0.41	1.18	−3.14	4.87	0.12	1	0.73

Shown are the model estimates, with standard errors (SE), lower and upper confidence intervals (CI), Chi-square of predictor (χ^2) and degrees of freedom (df). Analysis is based on N = 126 daily dyadic observations of migrants interacting with the peered-at item, on days where peering occurred. The conditional pseudo delta R^2 for this model was 0.99. For this analysis only data from the Suaq population were available.

2016c). Future studies should identify whether the learned and practiced knowledge is actually used in the migrants daily lives, and whether they also apply this knowledge when being alone without a demonstrator.

4.2 Peering target choice

In line with **prediction 2**, that migrants should preferably learn from those role models that have most knowledge of the local area, we found that in the highly sociable population of Suaq, migrants showed the highest peering rates at local philopatric females, followed by peering at local immatures and other adult unflanged males. Similar findings have been reported in wild male migrant vervet monkeys, that selectively learned from the philopatric females about how to open an "artificial fruit" (van de Waal et al., 2010). However, in the less sociable population of Tuanan, we found the opposite in the migrants' role model choice, as they showed the highest peering rates at adult unflanged males, followed by peering at local immatures and the least at philopatric females. Tuanan migrant males may not have many opportunities to peer at local females because females avoid long associations with males, (Kunz et al., 2021), and thus eventually also close proximity or feeding close to males.

In Suaq and Tuanan, males overall initiate and maintain associations with females more frequently than the females themselves. However, only in Tuanan, the Bornean population with the less productive habitat, prolonged associations with males led to an increase in fecal cortisol metabolite levels in females, suggesting that associations are stressful and costly for the females (Kunz et al., 2021). Accordingly, in Tuanan, females are also avoidant of unrelated females (van Noordwijk et al., 2012). These findings are thought to stand in direct connection with the less productive habitat in Borneo that causes increased levels of resource competition (Rijksen, 1978; van Schaik, 1999; Kunz et al., 2021). This argumentation is further supported by a study on the Tuanan orangutans showing that philopatric females will decrease their low level of gregariousness and social tolerance even more, when confronted with increased levels of competition due to the long-term effects of human-induced fires (Ashbury et al., 2022). Social intolerance towards migrants in such cases therefore most likely does not originate from strong social peer pressure

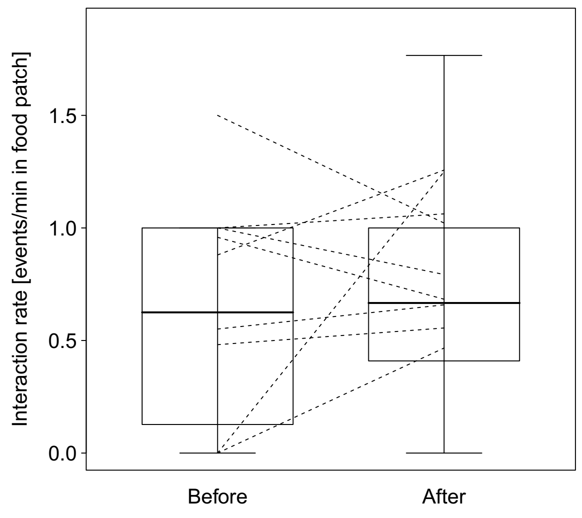

FIGURE 4
Migrants interaction rates per dyad with the peered-at item before and after the peering event at the Suaq population. Each dot represents the average rate per individual male depending on the condition (before and after). The median is indicated by the horizontal line, the upper and lower quartile are depicted by the box and the minimum and maximum values are shown by the whiskers.

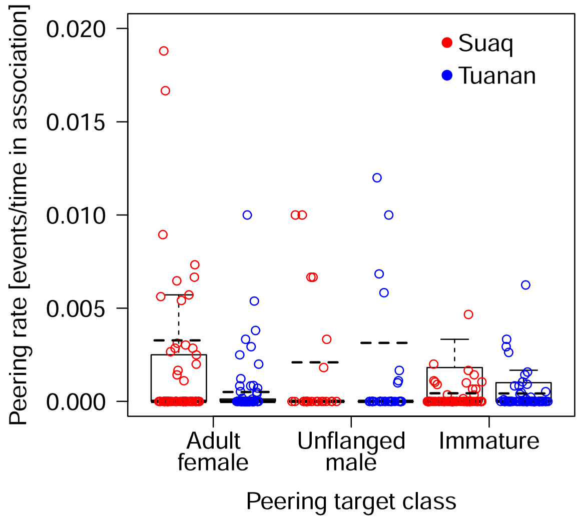

FIGURE 5
Migrants peering rates at specific peering target classes, controlled for the time in association. Each dot represents an average rate per individual male per peering target class, with the mean peering rate overall all males as a dotted black. The median is indicated by the horizontal line, the upper and lower quartile are depicted by the box and the minimum and maximum values are shown by the whiskers.

TABLE 4 The effect of the interaction between the role models' age-sex classes and site, on the migrants peering behavior analyzed using a GLMM with negative binomial family distribution.

Factor	Factor type	Estimate	SE	Lower CI	Upper CI	χ^2	*df*	*P*
Intercept	Intercept	−6.84	0.37	−7.69	−6.12	–	–	**< 0.001**
ClassTarget: Site	**Predictor**	–	–	–	–	15.02	2	**< 0.001**
FAI	Control	0.21	0.16	-0.14	0.5	0.58	1	0.45

The age-sex classes of the peering targets are: adult females, immatures and unflanged males. Shown are the model estimates, with standard errors (SE), lower and upper confidence intervals (CI), Chi-square of the interaction (χ^2) and degrees of freedom (df). Analysis is based on N = 2426 daily dyadic number of observations of migrants associating with peering targets of all age-sex classes, on days with and without peering, summed up quarterly per year. The conditional pseudo delta R^2 for this model was 0.12. Full information on *post-hoc* test (Tukey pair-wise comparisons) listed in Supplement A, Table S5.

(conformity) as it is seen in chimpanzees (Whiten et al., 2005) or vervet monkeys (van de Waal et al., 2013), but instead from direct competition over scarce resources. This is further supported by our result that at both sites, no peering was directed at flanged males, who are known to be less tolerant towards other males, likely because they compete over ecological resources and access to females with these males (Utami Atmoko et al., 2009).

It appears that migrants at Tuanan would then choose the "second best option to learn from", which are other adults like unflanged males or local immatures, who themselves (depending on their age, or the time already spent in the area) may have a substantial amount of local knowledge (Schuppli et al., 2016a). In general, immatures and unflanged males are the most sociable age sex classes in orangutans, which may favor relaxed associations in closer distance to each other (Mitra Setia et al., 2009). Previous studies showed that immature orangutans exhibit a growing interest in and tendency to peer at individuals other than their own mothers as they become older and more independent (Schuppli et al., 2016a). Furthermore, learning from immigrant males seems to be important for the immatures' skill acquisition, in particular for immature males (Ehmann et al., 2021). Therefore, unlike for the adult females, the benefits of learning from adult males may outweigh the costs of close associations with these males, especially for older and male immatures. These mutual benefits of knowledge exchange between adult males and immatures may promote close associations and peering between these classes. These patterns suggest, that in contrast to the established social learning theories, under conditions like in Tuanan, migrants seem to be forced to seek information from peering targets, that are unlikely to possess complete local information: unflanged males, who originated from different areas and immatures who have not completed their learning about the local area. This horizontal and oblique learning thus may bear the risk to obtain incomplete or even wrong information (Henrich & McElreath, 2003; Laland, 2004). These findings also demonstrate the males' selectivity and flexibility in their peering target choice. Similar flexibility in role model choice has been found in wild vervet monkeys, were migrants usually would copy local philopatric females, but do switch to copy dominant males when these males gain a much higher payoff in experimental food reward setups as females (Bono et al., 2018). This mental flexibility is generally already visible in immature orangutans, who, as they get older, increasingly learn from other individuals than the own mother (Schuppli et al., 2016c) and show sex-specific preferences for role models (Ehmann et al., 2021).

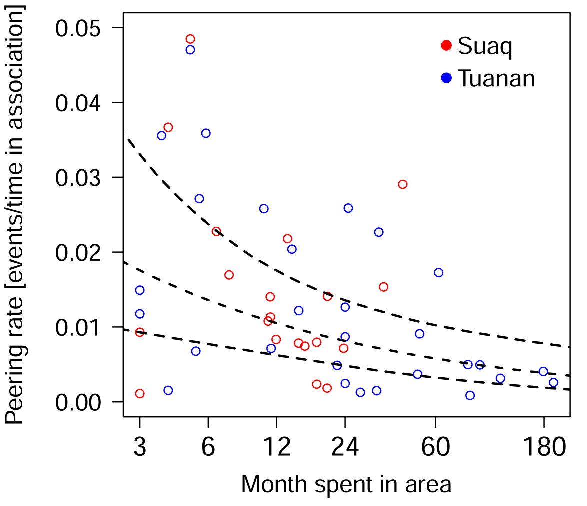

FIGURE 6
Migrants' peering rates, as a function of the number of absolute months spent in the study area until the day of the peering event, controlled for the time being in association with respective peering targets. Each dot represents the average rate per individual male per time point in area. The dashed line depicts the fitted model, with its confidence limits for the predictors as dotted lines.

4.3 Learning over time

In line with our prediction 3, we found that the migrants peering rate significantly decreased with increasing time the male had spent in the study area. Our results show that within the first 6 months after dispersal the migrants peering rates are particularly high. At this time, they likely still lack the majority of knowledge of the new area. Our results suggest that the subsequent gradual learning process can take multiple months, which may partly be due to the fact, that many food items are not available all year around. The drop in peering rates suggests that with increasing time spent in the area, the migrants' competency on local ecological increases and thus the need to learn decreases. These findings parallel how immature orangutans socially learn *via* peering, where with increasing age and competence, immature peering rates gradually decrease (Schuppli et al., 2016c). It has been estimated that over the course of their lifetime, Bornean and Sumatran orangutans peer approximately 9,000 and 38,000 times whereby the majority of all peering happens during immaturity

TABLE 5 The effect of the time spent in the area on the migrants peering behavior analyzed using a GLMM with Poisson family distribution.

Factor	Factor type	Estimate	SE	Lower CI	Upper CI	χ^2	*df*	*P*
Intercept	Intercept	−4.9	0.12	−5.1	−4.65	–	–	**< 0.001**
PresentMonthInArea	**Predictor**	−0.51	0.15	−0.8	−0.24	8.246	1	**< 0.001**
FAI	Control	0.32	0.19	−0.04	0.63	3.576	1	**0.087**
Site (Tuanan)	Control	0.02	0.24	−0.46	0.45	0.009	1	0.922

Shown are the model estimates, with standard errors (SE), lower and upper confidence intervals (CI), Chi-square of the predictors (χ^2) and degrees of freedom (df). Analysis is based on N = 149 daily dyadic observations of migrants associating with peering targets of all age-sex classes, on days with peering, summed up quarterly per year. The conditional pseudo delta R^2 for this model was 0.94.

(Schuppli and van Schaik, 2019). The results of our study suggest, that at least for the dispersing males, the "learning window" remains open over the course of their dispersal and the first years after it.

The fact that in immatures age has a strong effect on peering rates, raises the question whether age may also affect the peering rates of the males in this study. Unfortunately, because we do not know how old the males in our study are (because we have no information on their lives before their arrival at the study sites), we cannot test for age effects. However, when the males arrive in our study areas, their body sizes vary visibly (Schuppli and van Noordwijk, personal communication), which suggests that the males are of different ages. After their arrival, peering remains high for the first 6 months only, but then drops. Furthermore, in adult females, peering remains at very low levels throughout adulthood (Schuppli & van Schaik, 2019). Therefore, age effects alone are unlikely to bring about the peering patterns we found in the adult males.

5 General discussion

Our study represents a systematic analysis of adult migrant orangutans' observational social learning (measured in peering behavior) from local residents after dispersal. By examining the actual learning process (rather than focusing on the result of learning), we were able to identify details on the content and the role model choice. This stands in contrast to existing studies that have either focused on single migrants, and single behaviors of migrants or local conspecifics only, or within conditions that were altered experimentally. Furthermore, by including data collected over a study period of 30 years, we were able to track individual migrants peering behavior as a function of the time they had spent in the study area. We were thus able to cover the process of migrants' social learning, from their inferred arrival, followed by the chronological changes over time. The results parallel how immature orangutans socially learn during immaturity, but differ significantly in those areas (e.g. content and role model choice), that are specifically important for adult orangutans confronted with a new ecological environment after dispersal (see further down). Hence, the flexibility in wild adult orangutans' social learning might be key to counterbalance the costs of dispersal and to successfully adapt to a new habitat.

6 Conclusions

First: Dispersal seems to be a very disruptive phase for migrant orangutans. Although when compared to immatures, migrants show very low peering rates (Schuppli et al., 2016c), our results show that migrants temporarily resume peering as an observational form of social learning. Therefore, the existing ecological knowledge of migrants seems insufficient to thrive in the new habitat without social learning. Our results support the suggested third phase of social learning during primate development, which stated that migrants make use of social learning after dispersal to adapt to the new habitat (Whiten and van de Waal, 2018).

Second: With this study we add to the growing body of evidence that peering is a means of observational social learning. Observational social learning allows migrants to learn relevant local knowledge safely and efficiently without individual re-invention while benefitting from already existing knowledge instead. Not knowing what is edible and what is dangerous entails substantial risks and can lead to fatalities. Furthermore, individuals that are in possession of critical ecological information have a survival advantage (Kurman and Ronen-Eilon, 2004; Marzec, 2020). Follow-up studies should therefore examine to what extent the male's repertoires correlate with their fitness. Ultimately, since large bodied, long-lived and slow breeders like great apes, are especially challenged by sudden changes in their environment (next to dispersal, e.g. also due to habitat loss), relying on social learning for adaptation might be the most efficient way, since the adaptation through regular biological evolution would take too long for individuals to come up with advantageous traits just in time.

Third: In our study we found preliminary evidence that social tolerance might be the key to allow for relaxed learning situations. The local females in the less sociable species are known to avoid long associations with males (Kunz et al., 2021), which may decrease the migrants' opportunities to socially learn from them after arrival. At this point, it is not possible to identify the causes for this diminished learning opportunities. It is possible that either females and or males will increasingly grow more intolerant in times of scarcity and will therefore decrease physical closeness to each other to avoid direct resource competition by e.g. being in the same feeding patch. Overall, the successful long-term integration of a migrant might therefore critically depend on such tolerant ties with the local females.

Fourth: Being our most distant great ape relative, the study of the orangutans may reveal shared traits at the very basis of the Hominidae lineage some 12–14 million years ago (Locke et al., 2011). The ability for migrants to use observational forms of social learning after dispersal seems to be the ancestral state of the hominin lineage. The conditions at the time of this trait's emergence, must have selected for migrants that have the general ability to harvest the benefits of cultural knowledge through social learning from locals, but avoided the costs through competition in times of food scarcity. In the course of hominin evolution, this ability then developed further alongside the different species' social systems and formed the respective species' social learning biases; even eventually into the two most opposite extremes possibly, as it is seen now in modern-day chimpanzees *vs.* bonobos, which split approximately 2 million years ago (Prüfer et al., 2012). The human journey has always been a matter of migration and migrants are described to be exceptional people, in terms of versatility, resilience and adaptivity (Goldin et al., 2011); an ability we seem to share with our most distant great ape relative.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study on wild orangutans was strictly observational and non-invasive, and there was no interaction with our study animals in any way. The research protocols were approved by the Indonesian State Ministry for Research, Technology and Higher Education (RISTEK398/SIP/FRP/E5/Dit.KI/X|/2017) and complied with the legal requirements of Indonesia.

Author contributions

Design and development of models: JM, CSchu, AW, LK. Analyzing models: JM, LK. Design and conceptualizing research project: JM, AW, CSchu, CScha, MN. Data collection and preparation: JM, CSchu, MN, FL, JK, OW. Writing manuscript: JM. Review of the manuscript: all authors. Supported field work: AW, CSchu, CScha, MN, PR, SU. Supervision: AW, CSchu. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1158887/full#supplementary-material

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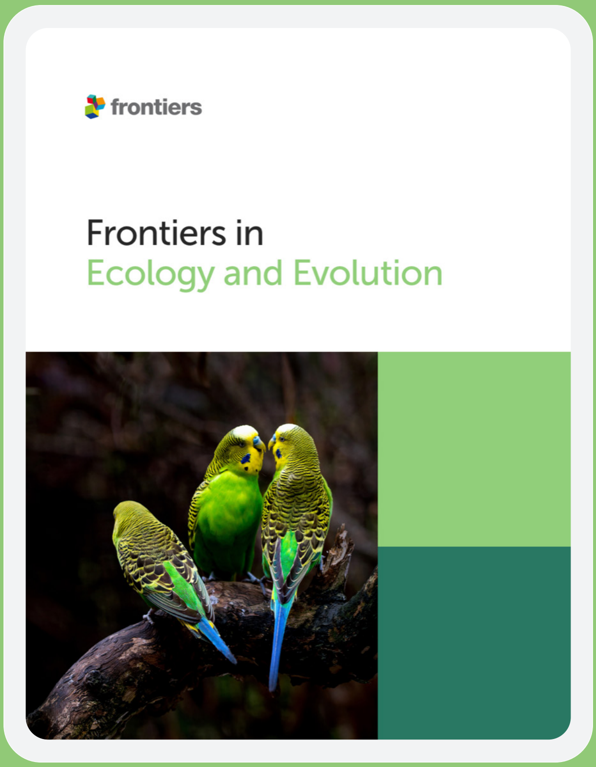
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