From Pavement to Population Genomics: Characterizing a Long-Established Non-native Ant in North America Through Citizen Science and ddRADseq

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Clarifying the population structure and introduction history of non-native species is essential to preventing and mitigating effects of invasion, yet the invasion history of many long-established non-native species remain poorly understood. This study characterized the population structure of one of the most commonly encountered urban ants in North America using public participant-collected specimens and double-digest restriction-site associated DNA sequencing (ddRADseq). Based on this genomic approach we confirm the identity of populations ranging across the continent as *Tetramorium immigrans* Santschi, and report low genetic diversity across its range, suggesting that this entire population resulted from the establishment of one single, or a few closely related ant colonies, approximately 200 years ago. The absence of strong population structure suggests that this ant's spread across North America has been repeatedly aided by human-assisted dispersal. We find no evidence of inbreeding in *T. immigrans*; this contrasts with the inbreeding and unicoloniality of many other invasive ants. These results demonstrate how collections-based public participatory science and emerging high-throughput sequencing techniques each have the potential to vastly improve the amount of data available to researchers. Together, these techniques can be deployed to address ecological and evolutionary questions, including questions about invasion biology, in non-model organisms. We suggest that this model is particularly well-suited for research on common taxa that are ecologically important but poorly understood.

Keywords: *COI*, high-throughput sequencing, invasive species, population genetics, social insects, *Tetramorium immigrans*, SNPs

INTRODUCTION

Studying the population genetics of non-model organisms, including non-native, urban and familiar "backyard" species, has historically been challenging because of the need for broad scale sampling and the limited availability of appropriate genetic markers. More recently, however, technological advances of the past decade—in both communications and genomics

(Klaper and Thomas, 2004)—have made it feasible to carry out population-level research with nearly any taxon, including invasive species. The introduction of non-native species as an unintended result of global trade increasingly threatens the environment and public health, as invasive species can reduce biodiversity, displace native fauna, serve as vectors for pathogens, and disrupt ecosystem services (Hulme, 2009; Ascunce et al., 2011; Bertelsmeier et al., 2017). Preventing establishment of invasive species and mitigating impacts of established invasives requires understanding species' origins and introduction routes, yet origins and introduction histories of many non-native species remain unknown (Ascunce et al., 2011).

Population genomic approaches, in particular, restriction site-associated DNA sequencing (RADseq) (Miller et al., 2007; Baird et al., 2008; Peterson et al., 2012), offer a cost-effective solution for surveying genome-wide single nucleotide polymorphism (SNPs) in species without a sequenced genome. These techniques have proven useful to understanding species' invasion histories (Dupuis et al., 2018; Leydet et al., 2018; Bors et al., 2019; Cohen and Privman, 2019) and as a non-destructive method for identification and monitoring (reviewed in Taylor and Gemmell, 2016). More generally, emergence of RAD-based techniques have led to wide adoption of these methods in ecological and evolutionary studies of insects (Bagley et al., 2017; Storer et al., 2017; Lower et al., 2018; Zhang et al., 2018; De Jesús-Bonilla et al., 2019), including ants (Moreau and Wray, 2017; Boyle et al., 2018; Avril et al., 2019; Smith et al., 2019), which rank among the most damaging and difficult to control invasive species (Rabitsch, 2011).

If there is an exotic species characteristic of urban habitats in the USA, it is certainly the pavement ant *Tetramorium immigrans* Santschi, whose specific epithet "*immigrans*" speaks to its history of long-distance dispersal and whose common name, "pavement ant," invokes the very essence of urban living: the paved environment. Like many other ants, this species forms large, subterranean colonies of many hundreds to thousands of workers (**Figure 1**). The generalist feeding habits of pavement ants make them exceptionally adaptable to new environments: they consume proteins, fats, and carbohydrates from plant and animal sources. Where food waste is common in cities, they are often the first animals to arrive at and break down discarded foodstuffs (Penick et al., 2015).

Even in their native range throughout Anatolia and the Caucasus, *T. immigrans* colonies commonly occupy human-modified habitats, nesting in pavement, rock walls, railways, and buildings as well as under stones along roadsides and in parks and gardens (Wagner et al., 2017). They favor warm, moist environments, especially during periods of high activity, although they easily tolerate cold and dry conditions during the winter phase of inactivity (Cordonnier et al., 2019). Pavement ants were likely first shipped unintentionally to the eastern USA in the early nineteenth century in soil ballast or in cargo (Steiner et al., 2008). The earliest confirmed records of this species in North America are from specimens collected in 1927 in the US North Atlantic states of Maryland, New Jersey, New York, Pennsylvania, and Washington D.C., and unconfirmed records exist from as early as 1748 (Wheeler, 1927; Brown, 1957). As

FIGURE 1 | Pavement ants, *Tetramorium immigrans*, are often encountered in urban and suburban areas throughout temperate North America. Here, workers from two colonies battle over territorial boundaries on the margins of a reservoir (Photo: Alex Wild).

of 2019, *T. immigrans* has been recorded in 39 states in the continental USA and three Canadian provinces (Guenard et al., 2017) (www.antmaps.org).

European pavement ants are not just widespread in North America, they are ubiquitous; multiple studies have found them to be the dominant ant species in urban environments (Pećarević et al., 2010; Buczkowski and Richmond, 2012; Lucky et al., 2014). Despite their prevalence in urban temperate North America, the identity and species limits of *T. immigrans* have long been uncertain (Wagner et al., 2017, 2018). Taxonomically, *T. immigrans*, along with several other morphologically similar species, belongs to the *Tetramorium caespitum* complex. A recent integrative taxonomic study of species boundaries in the complex which incorporated morphology, genes, and cuticular hydrocarbons (Wagner et al., 2017) paved the way for population-level study of *T. immigrans* and its close congeners. This research highlighted the possibility that populations of pavement ants across North America thought to be *T. immigrans* may represent multiple species. While one other species in the complex, the morphologically distinguishable Japanese *Tetramorium tsushimae* Emery, is present in a few midwestern and eastern states (Steiner et al., 2006), the possibility of other, cryptic European pavement ant species in North America remained.

Historically, restricted access to private property made biodiversity sampling in urban and suburban habitats challenging for researchers. However, public participants can collect in their own neighborhoods with ease, making a public participatory approach to sampling an easy solution (Dickinson et al., 2012; Ricciardi et al., 2017). Research partnerships with public participants offer one way to meet the extensive sampling needs of studies that assess population structure and draw inferences about widespread and common species (Dickinson et al., 2010). Common species, by definition, are relatively easy to find, even for non-specialists; nevertheless, they are often

woefully under-represented in museum collections. Whereas, observational data have been used effectively for mapping phenotypic, phenological, and geographic traits, the collection and preservation of physical specimens are necessary for genetic research (Pimm et al., 2015; McKinley et al., 2017). To date, a limited number of studies have combined these two approaches (Lyons et al., 2012; Kerkvliet et al., 2017; Ryan et al., 2019), and the value of public participatory research for characterizing introduction histories of non-native, urban species remains to be widely demonstrated empirically.

The goals of this study were to combine citizen science and population genomics to characterize identity and population genetic structure of the pavement ant, *T. immigrans*, in its US exotic range, and to reconstruct introduction history. We first used citizen-collected samples as the basis to (1) determine species identity. We used DNA sequence data from the mitochondrial gene *COI* to clarify whether US populations of *Tetramorium* belong exclusively to the species *T. immigrans* or to additional, morphologically similar species within the *T. caespitum* complex (excluding *T. tsushimae*). We then (2) quantified genetic diversity among populations in the North American range using genome-wide SNPs generated with ddRADseq. We anticipated low genetic diversity of this population would reflect an introduction scenario involving establishment of one or few closely related colonies, limiting the genetic diversity to only a fraction of that present in the native range. By contrast, high genetic diversity would indicate that North American populations descended from multiple lineages of *T. immigrans* and support a wave-like introduction scenario involving multiple colonies becoming established, possibly over an extended time frame, bringing with them breadth of diversity from across the native range. Finally, we used genomic data to (3) consider alternate dispersal patterns within the North American range. If expansion from the original introduction site occurred via local dispersal through mating flights—without the assistance of human transportation—we expected genetic diversity to increase with distance. Signatures of this history would be detectable as in the genetic differentiation between major continental regions, particularly between east- and west-coast populations. Conversely, lack of regional genetic differentiation would indicate that human-assisted dispersal helped the pavement ant become established across the continent.

MATERIALS AND METHODS

Specimen Collection and Preparation

Nearly all of the ant specimens used in this study were collected by participants—most of them school children—in the School of Ants citizen science project (SOA, www.schoolofants.org), between 2011 and 2013 (Lucky et al., 2014). SOA was designed to facilitate public engagement in scientific research; one goal was to use participant-collected samples of widespread ants for population genetic studies. Other goals included increasing interest and trust in science through public participation in scientific research (Vitone et al., 2016), facilitating monitoring of ant distributions in human-modified environments (Lucky et al., 2014), and encouraging the study of urban biodiversity.

All specimens were collected following the standardized SOA protocol (**Figure 2**, **Supplementary Material 1**), in which participants used cookie crumbs to attract ants, then collected ants in zip-top bags, froze them overnight, and mailed them to the SOA team. Upon receipt, ant specimens were identified to genus and species (wherever possible) and vouchers were stored at −18°C in 95% EtOH.

COI Sequencing

From hundreds of samples containing *Tetramorium* workers, 90 samples from 26 states were included in genetic analyses, representing the broadest possible US geographic range (**Figure 2**, **Supplementary Material 2**). To minimize unintentional sampling of ants from the same colony, specimens were collected from localities at least 1 km from any other sample, far beyond the estimated territory size of 42 m^2 in a single colony of *T. immigrans* (Brian and Elmes, 1974). DNA was extracted destructively using the Qiagen DNeasy blood and tissue kit (QIAGEN, Valencia, CA), according to standard protocol with the only change being the final elution volume of 75 μL DNA for each sample. A 658 bp fragment of the mitochondrial *COI* gene, commonly used as a "barcoding" gene, was amplified using primers LepF1 (5′-ATT CAA CCA ATC

FIGURE 2 | (Top) Pavement ant specimens were collected by public participants, most of them children, through the public science project, the School of Ants (Photo: Jonas Stenstrom). **(Bottom)** Map of US *Tetramorium immigrans* specimen sampling localities; colors correspond to geographic regions.

ATA AAG ATA TTG G-3$'$) and LepR1 (5$'$-TAA ACT TCT GGA TGT CCA AAA AAT CA-3$'$) using standard PCR conditions (Folmer et al., 1994).

COI Data Processing

To contextualize North American *T. immigrans* among native range samples, we also took advantage of additional, although not fully overlapping (348 bp), *COI* sequences of European *T. immigrans* from previous study (Schlick-Steiner et al., 2006). We built a TCS haplotype network (Clement et al., 2000) using the combined European and our own *COI* data in the program POPART v1.7 (Leigh and Bryant, 2015).

ddRADseq Sequencing

Double digest Restriction-site Associated DNA sequencing (ddRADseq) library preparation followed (Peterson et al., 2012) using the 78 samples that successfully generated *COI* sequences. In addition, to compare inter- vs. intra-colonial levels of genetic diversity and test for inbreeding, we included 16 workers from a single colony in North Carolina. In brief, the rare EcoRI and common MseI restriction sites were used for restriction enzyme digestion. Illumina sequencing adaptors and custom barcodes between 8 and 14 bases in length, which varied by at least four bases (Parchman et al., 2012), were ligated onto the sticky ends and PCR-amplified using primers for Illumina sequencing adaptors. Amplified digestion-ligation products were pooled into equal volumes for sequencing. Gel-based size selection for 200–500 base pair fragments and purification of the sequencing library using Agencourt AMPure XP beads (Beckman Coulter) were performed at the UF ICBR core-sequencing laboratory (http://www.biotech.ufl.edu), and sequenced using Illumina NextSeq500 high-output flow cell for a shared 150-cycle sequencing run.

RADseq Data Processing

Illumina adaptor sequences were automatically trimmed from all reads by Illumina's BaseSpace software. All subsequent bioinformatics steps were conducted using Stacks v. 2.3e (Catchen et al., 2013). Raw Illumina reads were demultiplexed using *process_radtags*, quality controlled (-c -q -r) and trimmed to 100 base pairs (-t). The Stacks program *denovo_map.pl* was used for *de novo* assembly, locus discovery, and genotyping. A minimum of three mismatches was allowed between stacks during loci assembly (-M 3), and an allowance of two mismatches was permitted between stacks (-n 2). Finally, the Stacks *populations* program was used to identify loci violating Hardy-Weinberg equilibrium (-hwe), which were subsequently removed via the blacklist option (-B). The remaining loci were then filtered to include only loci present in all four populations (-p 4), and in 90% of the individuals (-r 0.9), for downstream analyses.

Basic population and summary statistics were calculated using Stacks populations *sumstats.summary* output, the R packages *adegenet* v2.1.1 (Jombart and Ahmed, 2011) and *hierfstat* v0.04-22 (Goudet, 2005). To infer population structure the Bayesian-based clustering analysis, STRUCTURE v2.3.4, was used (Pritchard et al., 2000). We tested the support for 1 through 5 genetic clusters ($K = 1–5$) and ran 10 replicates per K with 100,000 generations burn-in followed by 500,000

MCMC repetitions, using default settings. To infer the most likely number of unique genetic clusters we used the ΔK method (Evanno et al., 2005), implemented in STRUCTURE Harvester (Earl, 2012). The STRUCTURE plots were visualized using *pophelper* v2.2.9 (Francis, 2017). We also analyzed the SNPs data using the discriminant analysis of principle components (DAPC), which has fewer marked assumptions than STRUCTURE, using *adegenet*. The most probable number of clusters for DAPC was determined using sequential *k-means* clustering using the *find.clusters* function and the associated Bayesian Information Criterion (BIC). DAPC plots were made for individuals under the most probable cluster membership and labeled by U.S. geographic origins (Northeast, Mid-Atlantic, Midwest, and Western). Hierarchical analysis of molecular variance (AMOVA) was conducted to detect differences among populations using the R package *poppr* (Kamvar et al., 2014).

RESULTS

Mitochondrial Gene *COI*

Out of the 90 specimens that were tested, 14 failed to amplify; this was presumed to be due to DNA degradation during shipping. We were able to verify that all specimens morphologically identified as *T. immigrans* in this study were indeed this species using DNA barcode authentication of samples. No other cryptic species in this complex were found among these samples and very little genetic difference was found within the US. Comparison of US and European populations using a short fragment of *COI* recovered all US haplotypes as identical to the most commonly collected haplotype from Europe (**Figure 3**).
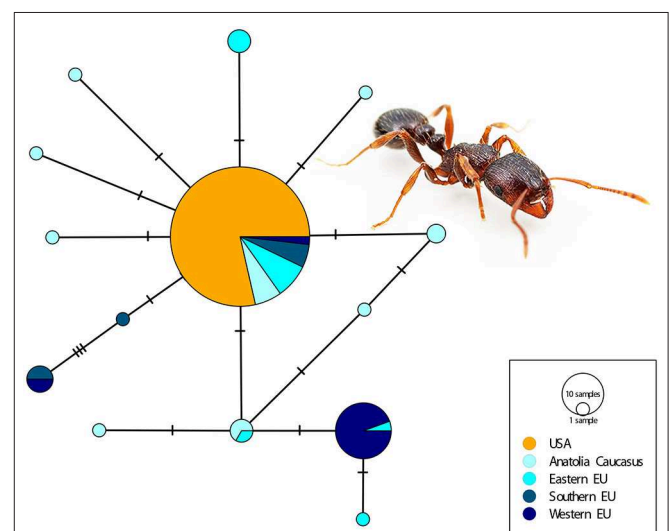

FIGURE 3 | TCS haplotype network of *Tetramorium immigrans* from the European and North American populations generated using a 348 bp *COI* fragment, with the following geographic designations: Anatolia/Caucasus (Armenia, Russia, Turkey), Eastern Europe (Bulgaria, Croatia, Czechia, Hungary, Romania), Southern Europe (Greece, Italy, Spain), Western Europe (Austria, France), and USA (Photo: Alex Wild).

Genome-Wide SNPs

The success rate of sequencing citizen-collected samples was high (98% success) with ddRADseq, on average, there were 970,000 $\pm$ 36,000 SE reads per individual. After stringent filtering 2,255 unlinked SNPs were retained for downstream analyses. The North American population of *T. immigrans* has little population structure: across the entire sampled range overall F_{ST} was low ($F_{ST} = 0.0150$, **Table 1A**). Within the one North Carolina colony for which 16 nestmates were sampled, we found near-identical genotypes ($F_{ST} = 0.001$, **Table 1A**). The inbreeding coefficient (F_{IS}) for the North Carolina colony was -0.4706, while the continent-wide F_{IS} was 0.0376 (**Table 1A**). Pairwise F_{ST} values between the four geographic regions were also low, ranging from 0.0056 to 0.0297 (**Table 1B**). The majority (94.82%) of genetic variation was found among individuals, rather than between regions as indicated by AMOVA (**Table 2**).

The optimal number of populations supported by STRUCTURE analysis was $K = 2$ (**Figure 4**), with Midwest and Mid-Atlantic samples grouped together while Western and Northeast populations had greater similarity to each other. For $K = 3$, additional structure was detected with two Northeastern individuals from Vermont having shared ancestry assigned primarily to this third cluster (**Figure 4**). The optimal number of clusters detected using *k-means* clustering was also

TABLE 1 | **(A)** Summary statistics of the *Tetramorium immigrans* SNPs data **(B)** pairwise F_{ST} based on the four geographical regions (WE, Western; MA, Mid-Atlantic; MW, Midwest; NE, Northeast).

	Observed heterozygosity	Expected heterozygosity	Gene diversity	F_{ST}	F_{IS}
(A)					
Within colony ($n = 16$)	0.1717	0.1168	0.1169	0.0010	-0.4705
Overall ($n = 79$)	0.1967	0.2043	0.2074	0.0150	0.0376

	MA	MW	NE
(B)			
MW	0.0161		
NE	0.0244	0.0208	
WE	0.0297	0.0229	0.0056

TABLE 2 | Analysis of molecular variance (AMOVA) of the *Tetramorium immigrans* SNPs data using clustering between the four geographical regions.

Source of variation	df	Variance component	% Total variation	Φ-statistics	p-value
Between regions	3	5.37	1.15	0.05	0.01
Between samples within region	75	18.87	4.03	0.04	0.01
Within samples	79	444.08	94.82	0.01	0.01

two, corresponding to the lowest BIC value (**Figure 4**). DAPC was subsequently carried out using the optimal two clusters and retained three discriminant eigenvalues, which separated Midwest population from the other three regions (**Figure 5**).

DISCUSSION

Species Identity

The most common, widespread pavement ant in North America is unequivocally *T. immigrans*, as evidenced by *COI* and ddRADseq data sampled from colonies sampled across North America. The near absence of genetic diversity ($\sim$0.0%) revealed in our study is consistent with previous, small-scale studies that found 0.0–1.2% *COI* sequence divergence for *T. immigrans* in its native range (Schär et al., 2018). We found no evidence of other *T. caespitum* complex species beyond the known presence of *T. tsushimae* (Steiner et al., 2006), which was readily distinguishable from *T. immigrans* based on morphology and geographic distribution.

Population Structure

Despite having been established in North America since at least the 1800s, little genetic divergence exists among populations of *T. immigrans* within the non-native range. Whether this is a result of selection or reflects the limited diversity present in the population upon establishment is unclear. We recovered only a few genetic variants from across a vast geographic range, and thousands of sites across the genome. *COI* sequences, for example, were nearly identical across samples; among three haplotypes identified, each differed from the others by only a single SNP. When compared with other European samples, the USA specimens clustered with the most common haplotype found throughout different regions of Europe (**Figure 3**).

Genomic data also indicate that pavement ants comprise a single, largely undifferentiated population across North America, with close examination revealing differences in structure that varied depending upon the analytical method used. In both STRUCTURE and DAPC, SNP data provided support for the existence of two weakly differentiated *T. immigrans* clusters (**Figures 4, 5**). In the $K = 2$ scenario, most samples were assigned to one cluster, Cluster 1, that was commonly encountered in all four geographic regions, with Cluster 2 represented only in a small subset of samples from the Northeast and West. Additional, minor, genetic sub-clusters were identified when $K = 3$. By contrast, DAPC analyses identified the composition of Midwestern samples as distinct from the other three geographic regions (**Figure 5**). This overall lack of strong genetic structure was also confirmed by AMOVA, as genetic variation among individuals far surpassed variation between assigned geographical regions (**Table 2**). Surprisingly, this low diversity appears not to result from inbreeding, as indicated by a low inbreeding coefficient (F_{IS}) among the 16 nestmates from a single North Carolina colony (**Table 1A**). While the patterns of nestmate variability could be underestimated by only examining a single colony, when combined with the lack of strong population structure across the US, they suggest that the entire North American population is likely descended from either
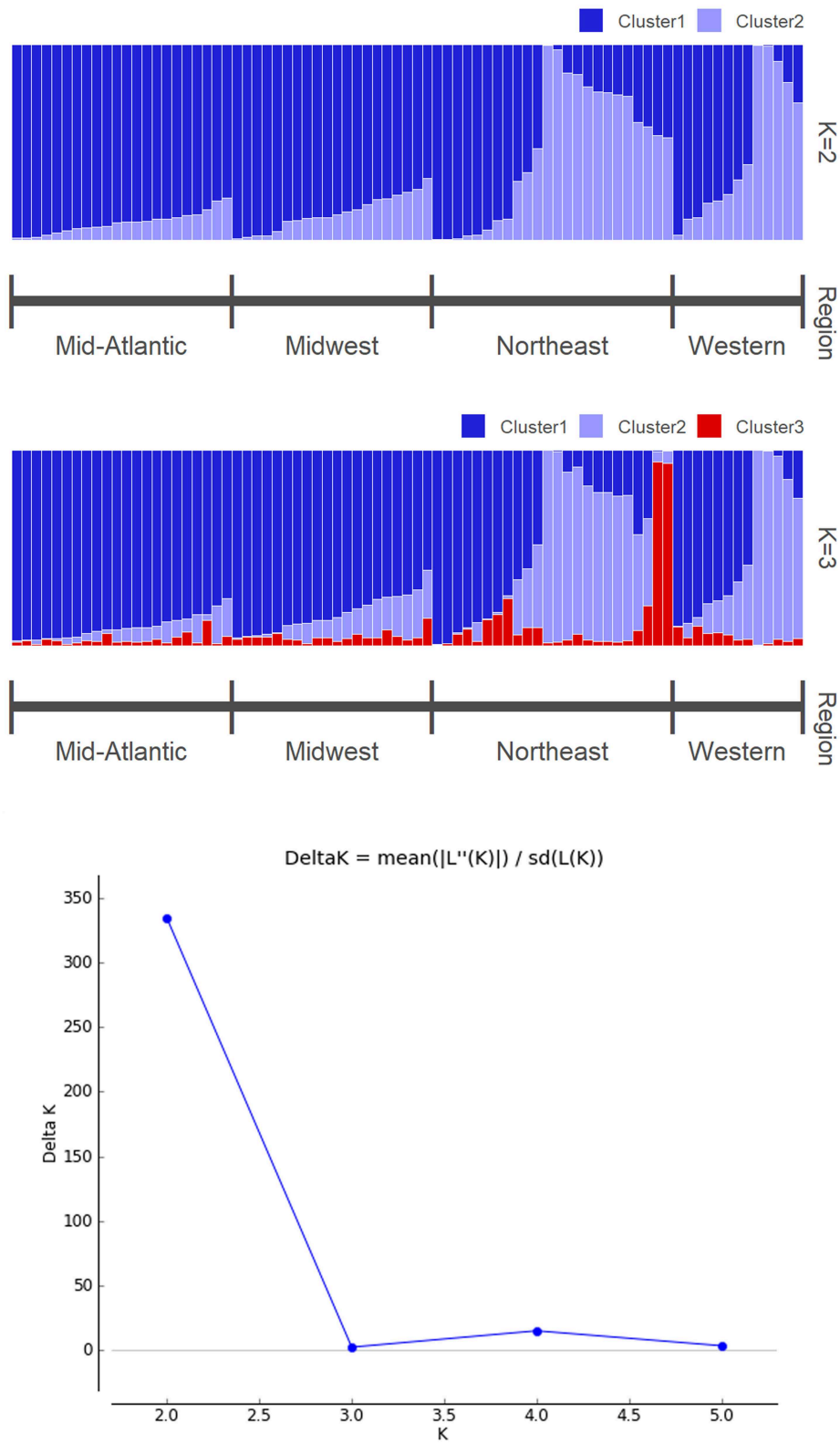
FIGURE 4 | (Top) Molecular assignments of $K = 2$ of *Tetramorium immigrans* based on analysis of SNPs using STRUCTURE. **(Middle)** Alternative molecular assignment of $K = 3$. **(Bottom)** Delta K value showing the most likely number of clusters indicated by STRUCTURE HARVESTER.
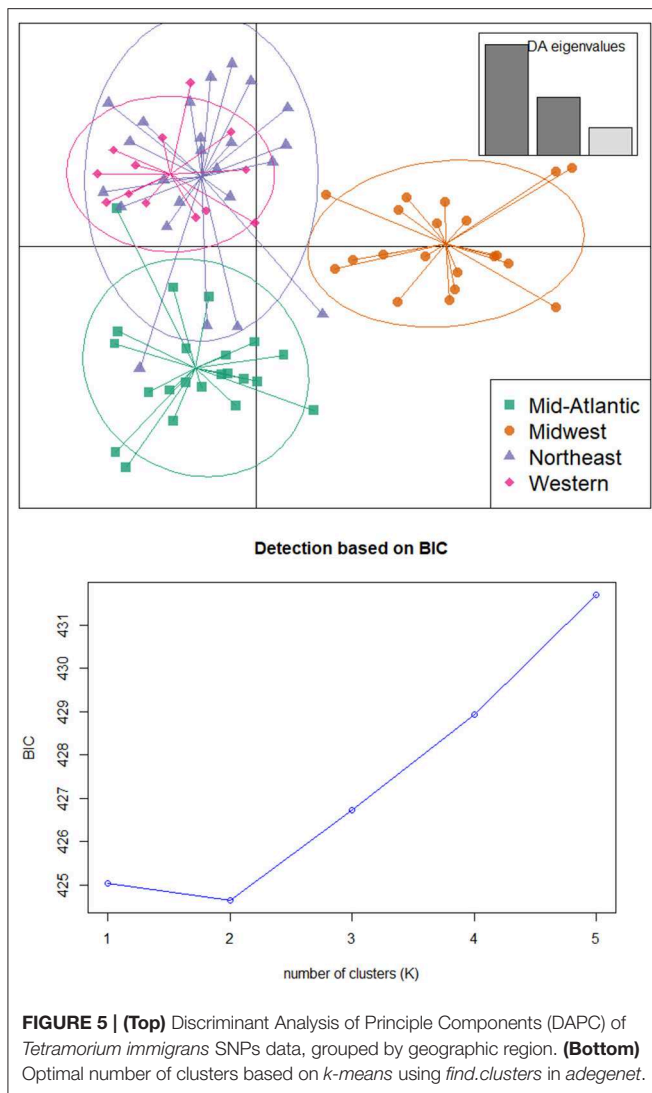
FIGURE 5 | (Top) Discriminant Analysis of Principle Components (DAPC) of *Tetramorium immigrans* SNPs data, grouped by geographic region. **(Bottom)** Optimal number of clusters based on *k-means* using *find.clusters* in *adegenet*.

a single introduction or multiple introductions from a single, genetically homogeneous lineage.

Our data do not allow us to distinguish between a single-colony and a multiple-colonies-from-a-single-lineage introduction scenario, but the presence of a rare social parasite, *Tetramorium atratulum* (Schenck) in the non-native range may provide an informative clue. This workerless ant is an obligate parasite of *T. immigrans* and is found at low densities in both the native and non-native range of its host (Dash and Sanchez, 2009). *Tetramorium atratulum* is fed and cared for exclusively by *T. immigrans* workers, which do not recognize this interloper as a threat. It seems unlikely that a viable population of this parasite could have become established in North America as a result of a single-colony host introduction. Instead, its presence suggests that a pulse of introduction and establishment of closely related pavement ant colonies may have been more likely. Future studies on the population genetics of *T. atratulum* could prove to be especially illuminating as to the introduction history of both this parasite and host species.

Despite the fact that the establishment of the European pavement ant in North America precedes that of other invasive ants by a century, the low genetic diversity in this species is nonetheless similar to that of other invasive ant species (Tsutsui and Suarez, 2003; Ascunce et al., 2011; Eyer et al., 2018). The combination of outbreeding and the genetic bottleneck experienced during founding resulted in a widespread population today that largely lacks genetic structure. The continent-wide range expansion from the initial site of establishment was likely facilitated by human-assisted dispersal, as we found no evidence of geographic isolation across the non-native range.

Invasion Biology of Pavement Ants

The genetic uniformity of *T. immigrans* across North America is surprising, considering its reproductive biology, which differs from that of other widespread and ecologically dominant non-native ant species. Low genetic diversity in this outbreeding species appears to be a result of the severely limited genetic diversity of the initial population upon establishment rather than inbreeding or clonal reproduction. Invasive ant success is often associated with unicoloniality, where colonies reach massive size and density as a result of multi-queen (polygynous) and multi-nest (polydomous) colonies that display no intercolonial aggression (Tsutsui and Suarez, 2003). Unicolonial populations often possess extremely low genetic diversity as a result of introduction bottlenecks and inbreeding (Tsutsui et al., 2003; Ascunce et al., 2011; Eyer et al., 2018) and in some cases clonal reproduction depresses diversity even further (Foucaud et al., 2009; Pearcy et al., 2011).

Tetramorium immigrans is widespread in paved habitats throughout most of temperate North America and maintains large and often competitively dominant colonies; however, each colony possesses only a single, outbreeding queen and has distinct territorial boundaries that workers aggressively defend against non-nestmates (Bruder and Gupta, 1972). By contrast, *T. tsushimae*, the other US introduced pavement ant in the *T. caespitum* complex, is polygynous. This difference in reproductive biology likely contributes to the competitive dominance of *T. tsushimae* where both species co-occur (Reuther, 2009; Trager, pers. comm.); whether this more recently introduced pavement ant will replace *T. immigrans* entirely remains to be seen.

Little is known about where, specifically, *T. immigrans* first became established in the US and precisely when this occurred. Brown (1957) suggests an introduction in or before the early 1800s. Given this timeline, it is surprising that stronger population structure does not exist in the non-native range. The yellow crazy ant, *Anoplolepis gracilipes* Smith, became structured and differentiated in its Australian exotic range within a matter of decades (Gruber et al., 2013). Reproductive biology may offer some insight into these differences. *A. gracilipes* colony foundation is dependent, with new colonies "budding" by fission from a parent colony; resulting in restricted gene flow and outbreeding distance. In contrast, a single mated *T. immigrans* queen independently establishes a new nest without attendant workers, a process known as independent colony foundation (Bruder and Gupta, 1972). Outbreeding and the mating flights

of *T. immigrans* sets them apart from many other well-studied invasive ants and may explain why minimal population structure exists within the US.

The results of this study show a higher genetic diversity among colonies than within a single colony (**Table 1A**), confirming that, at least in the region of this single-colony sample (North Carolina), *T. immigrans* does not form supercolonies in the exotic range. If not unicoloniality, what, then, can account for the success of *T. immigrans*? It seems likely that the combination of habitat suitability and the almost complete lack of competition from other North American ant species in the human-built environment (that is, open, gravelly, and well-drained sites), has aided in the expansion of *T. immigrans* (Cordonnier et al., 2019). The development of roads and railways, as well as increasing urbanization, have effectively rolled out the red carpet for this species. The generalist feeding habit of *T. immigrans*, combined with a preference for habitats sharing the qualities of paved environments in temperate climates, created a suitable and largely competitor-free niche for *T. immigrans* after its introduction into early 1800s North America. Urban *T. immigrans* have been found to have carbon-13 levels similar to those in human foods, to a greater extent than co-occurring ant species, suggesting that *T. immigrans* is exquisitely capable of taking advantage of human-produced (and discarded) food resources (Penick et al., 2015).

Although *T. immigrans* is most often associated with urban areas and paved surfaces, populations of this species have also become established in undisturbed, unpaved habitats in the western US, in California, Nevada, Utah, and Idaho, where the incursion of this species into intact ecosystems is associated with reduction of local ant species richness (Ward, pers. comm.). As the pavement ant is not strictly limited to the built environment, research on this species' ability to compete with native ants would be of interest. Further studies comparing the intraspecific genetic variations and behavior of this species in both its native and non-native ranges would shed more light on the mechanisms leading to the success of pavement ants, and indeed other synanthropic species, in the Anthropocene.

CONCLUSION

In this study we have shown that samples of ants collected by public participants can be incorporated into population genomic studies to answer otherwise intractable questions. Our results confirm that the commonly encountered pavement ant in North America is largely limited to a single species, *T. immigrans,* which ranges across the USA and southern Canada. Weak population structure among geographically distant populations suggests that this species' origins can be attributed to the establishment of one or a few genetically similar colonies that successfully proliferated and dispersed over two centuries. By synergistically combining citizen-collected specimens and molecular evolutionary studies, we offer an example of a feasible way to take citizen science into the genomic era. Partnerships between these two disciplines can both engage the public in the tools and methods of modern evolutionary biology and, simultaneously, permit evolutionary

biologists to address questions on a scale that would be difficult to impossible without the assistance of public participants. Relatively inexpensive genomics and informatics tools have the potential to powerfully enhance the research and educational impacts associated with the rich diversity of questions, subjects, and modes of participation that citizen science projects address (Pocock et al., 2017; Ryan et al., 2019). We hope the results presented here encourage collaboration among scientists and educators as this proven framework for rapid and cost-effective population genomics can be used to characterize other non-model species.

DATA AVAILABILITY STATEMENT

All *COI* sequences are deposited on Genbank accession numbers KT339872—KT339947, demultiplexed ddRADseq data are stored at Short Read Archive (SRA) under BioProject ID PRJNA539993.

AUTHOR CONTRIBUTIONS

AL, TV, and RD conceived and designed the study and collected the data. TV, YZ, AP, and CS analyzed the data. JH and SM contributed materials and analysis tools. AL, TV, and YZ wrote the paper with contributions from all authors.

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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. 2019.00453/full#supplementary-material

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