



Evolutionary Responses of *Drosophila melanogaster* Under Chronic Malnutrition

Muhammad Ahmad^{1,2,3†}, Erin S. Keebaugh^{2,3†}, Muhammad Tariq^{1*} and William W. Ja^{2,3*}

¹ Department of Biology, SBA School of Science and Engineering, Lahore University of Management Sciences, Lahore, Pakistan, ² Department of Neuroscience, The Scripps Research Institute, Jupiter, FL, United States, ³ Center on Aging, The Scripps Research Institute, Jupiter, FL, United States

OPEN ACCESS

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*Correspondence:

Muhammad Tariq
m.tariq@lums.edu.pk
William W. Ja
wja@scripps.edu

[†] Shared co-first authorship.

Specialty section:

This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 29 October 2017

Accepted: 04 April 2018

Published: 19 April 2018

Citation:

Ahmad M, Keebaugh ES, Tariq M and
Ja WW (2018) Evolutionary
Responses of *Drosophila*
melanogaster Under Chronic
Malnutrition. *Front. Ecol. Evol.* 6:47.
doi: 10.3389/fevo.2018.00047

Drosophila species have successfully spread and adapted to diverse climates across the globe. For *Drosophila melanogaster*, rotting vegetative matter provides the primary substrate for mating and oviposition, and also acts as a nutritional resource for developing larvae and adult flies. The transitory nature of decaying vegetation exposes *D. melanogaster* to rapidly changing nutrient availability. As evidenced by their successful global spread, flies are capable of dealing with fluctuating nutritional reserves within their respective ecological niches. Therefore, *D. melanogaster* populations might contain standing genetic variation to support survival during periods of nutrient scarcity. The natural history and genetic tractability of *D. melanogaster* make the fly an ideal model for studies on the genetic basis of resistance to nutritional stress. We review artificial selection studies on nutritionally-deprived *D. melanogaster* and summarize the phenotypic outcomes of selected animals. Many of the reported evolved traits phenocopy mutants of the nutrient-sensing PI3K/Akt pathway. Given that the PI3K/Akt pathway is also responsive to acute nutritional stress, the PI3K/Akt pathway might underlie traits evolved under chronic nutritional deprivation. Future studies that directly test for the genetic mechanisms driving evolutionary responses to nutritional stress will take advantage of the ease in manipulating fly nutrient availability in the laboratory.

Keywords: adaptation, artificial selection, evolved traits, experimental evolution, malnutrition, nutritional deprivation, PI3K/Akt, starvation

INTRODUCTION

The colonization of diverse ecological niches exposes organisms to varied environmental stresses. These stresses exert selection pressures which might lead to changes in physiology and behavior. Changes that enhance organismal fitness can result in adaptive evolution, a process by which selection acts on heritable changes in the genetic architecture of a population. Thus, studies on how organisms respond to chronic stressors provide valuable insights on adaptation.

Recent advances have uncovered varied responses to stress in different organisms (Badyaev, 2005). Among the limited number of model systems that have been established for these studies, the vinegar fly, *Drosophila melanogaster*, remains one of the most tractable. Adaptation to diverse environmental conditions underlies the successful global spread of *Drosophila* (Stephan and Li, 2007), facilitating its dissemination across multifarious ecological habitats (Keller, 2007). Physiological and behavioral responses have helped the fly adapt to and survive in conditions

encompassing a wide range of humidity (Matzkin et al., 2007; Ahuja et al., 2012; Griffin et al., 2016), oxygen concentration (Romero et al., 2007; Zhou et al., 2007), temperature (Bochdanovits and de Jong, 2003; Trotta et al., 2006), and nutrient availability (Kolss et al., 2009). Reproducing diverse environments in the laboratory has revealed how various parameters influence different fly traits, but the molecular mechanisms underlying adaptation to varied habitats are still unclear.

Due to the ease in which the fly diet can be manipulated in the laboratory, nutrition has been a major focus in studies of environmental factors that affect stress-induced acquired and heritable traits (Rion and Kawecki, 2007). In the wild, *Drosophila* larvae develop in ephemeral habitats such as rotting vegetative matter, resulting in rapid changes to food quantity and quality. Nutritional stress can include starvation due to food deprivation and malnourishment due to nutrient imbalance or depletion. Nutritional scarcity is further exacerbated by competition as greater larval density depletes scant resources. Moreover, other species share habitats with *Drosophila*, resulting in interspecies competition that further contributes to nutritional stress.

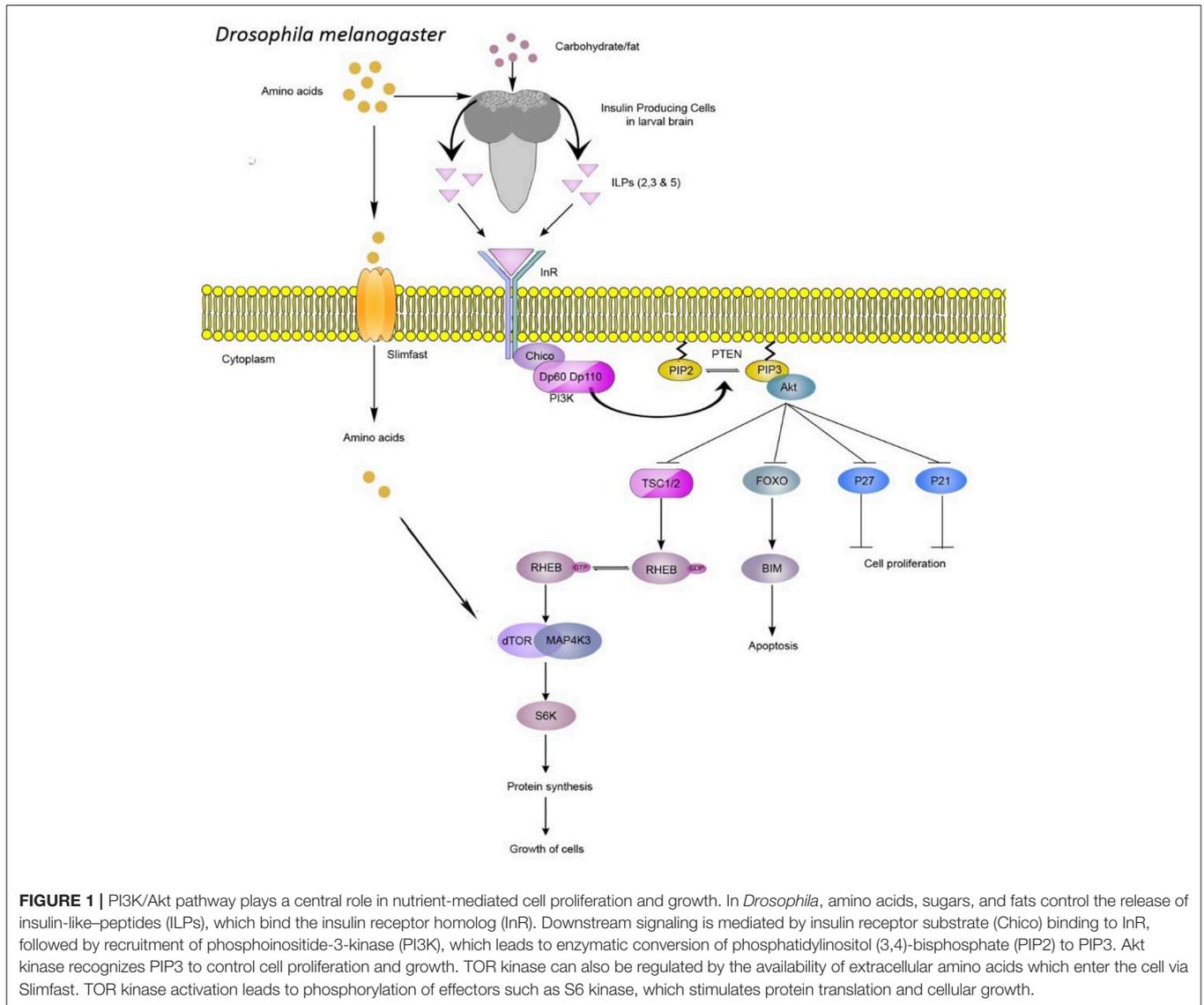
Drosophila larval populations selected on nutrient-poor food exhibit various behavioral and physiological changes (Kolss and Kawecki, 2008; Kolss et al., 2009; Vijendravarma et al., 2011, 2012a,b, 2013; Vijendravarma and Kawecki, 2013). These changes include altered foraging behavior, accelerated development, reduced body weight, and smaller body size (Kolss and Kawecki, 2008; Kolss et al., 2009; Vijendravarma et al., 2011, 2012b, 2013). These phenotypes are maintained when selected populations are subsequently raised on a richer diet, suggesting a genetic basis for these traits (Kolss and Kawecki, 2008; Kolss et al., 2009; Vijendravarma et al., 2012b, 2013). Interestingly, morphological changes in response to nutritional stress phenocopy mutants of PI3-kinase (PI3K)/Akt signaling, a pathway known to be responsive to nutritional status (Chen et al., 1996; Montagne et al., 1999; Oldham et al., 2000; **Figure 1**). Hence, under prolonged malnutrition, we hypothesize that fly nutrient-sensing pathways might be under selective pressure.

Genome-wide expression profiles revealed overlapping changes in gene transcript levels between TOR kinase null mutants and starved control animals (Li et al., 2010). Hence, the effects of short-term nutritional stress might be partially mediated by reduced TOR signaling. Despite these studies on acute starvation, it remains unknown whether PI3K/Akt signaling is also altered under chronic nutritional stress. Future studies using targeted genomic approaches might reveal the selection of this pathway and unravel malnutrition-mediated evolutionary responses that underlie tolerance to nutritional stress. Here, we review the studies that have evolved phenotypic responses under chronic malnutrition and highlight the possibility that the PI3K/Akt pathway is under selection during prolonged nutritional adversity in flies. These perspectives inform our understanding of the molecular basis of the physiological and behavioral changes associated with adaptation to malnutrition.

BODY SIZE AND WEIGHT

Evolved body size is associated with available nutrition. Flies selected for 29 generations on low concentration sugar and protein medium exhibit a number of adaptive consequences, including smaller body size and reduced weight (Kolss et al., 2009). Conversely, a quasi-natural selection experiment using a protein-rich diet favored large flies with greater dry mass and reduced lipid content compared to controls (Kristensen et al., 2011). Interestingly, mutations in components of the PI3K/Akt pathway impede larval growth and also reduce body size. Genetic manipulations of ILPs, InR, Chico, PI3K, Akt, TOR, S6K, FOXO, and Slimfast (**Figure 1**) have all been found to reduce body weight or size in larvae or adults (Böhni et al., 1999; Montagne et al., 1999; Oldham et al., 2000; Zhang et al., 2000, 2009; Britton et al., 2002; Colombani et al., 2003; Hietakangas and Cohen, 2007; Mattila et al., 2009), mimicking development under poor nutritional conditions (Kolss et al., 2009; Vijendravarma et al., 2011, 2012a). Such parallels in the literature bolster our hypothesis that the nutrition-sensing PI3K/Akt pathway might be under selective pressure during prolonged malnutrition. Changes in body size or weight might be associated with multiple factors, including the critical size required for initiation of metamorphosis (Tennesen and Thummel, 2011). Fly growth during development is characterized by two distinct phases: (1) a pre-critical-weight phase that can vary in time depending on the nutritional conditions and (2) a post-critical-weight phase where nutritional stress can no longer delay pupariation. During the pre-critical-weight phase, insulin signaling affects development time but not body size whereas, in the post-critical-weight period, it affects body size and not development time (Mirth et al., 2005; Shingleton et al., 2005). Hence, any changes in critical weight threshold can have a direct impact on both development time and body size. Interestingly, flies selected under chronic malnutrition develop more quickly (Kolss et al., 2009), possibly due to reduced critical size threshold (Vijendravarma et al., 2012a). Although mutations in PI3K/Akt pathway components result in a developmental delay (Zhang et al., 2000; Rulifson et al., 2002; Layalle et al., 2008), shortened development time in malnutrition-selected populations might be caused by altered prothoracicotrophic hormone signaling, which has been proposed to regulate critical size upstream of TOR function (Layalle et al., 2008).

Once critical weight is attained, the prothoracic gland (PG) induces pupariation through release of ecdysone (Mirth et al., 2005). Hence, a lowered critical weight threshold results in earlier release of ecdysone. PG cells grow in size due to an organ-specific augmentation in PI3K/Akt signaling that results in strikingly reduced critical weight culminating in an increase in the basal level of ecdysteroid biosynthesis (Colombani et al., 2005; Mirth et al., 2005). This consequently results in accelerated development and reduced body size due to a decrease in cell number (Colombani et al., 2005; Mirth et al., 2005). Furthermore, it was shown that the growth rate of flies with an enlarged PG was dependent on nutritional conditions (Mirth et al., 2005). Together, these results indicate that PI3K/Akt-mediated growth of the PG negatively regulates the developmental time

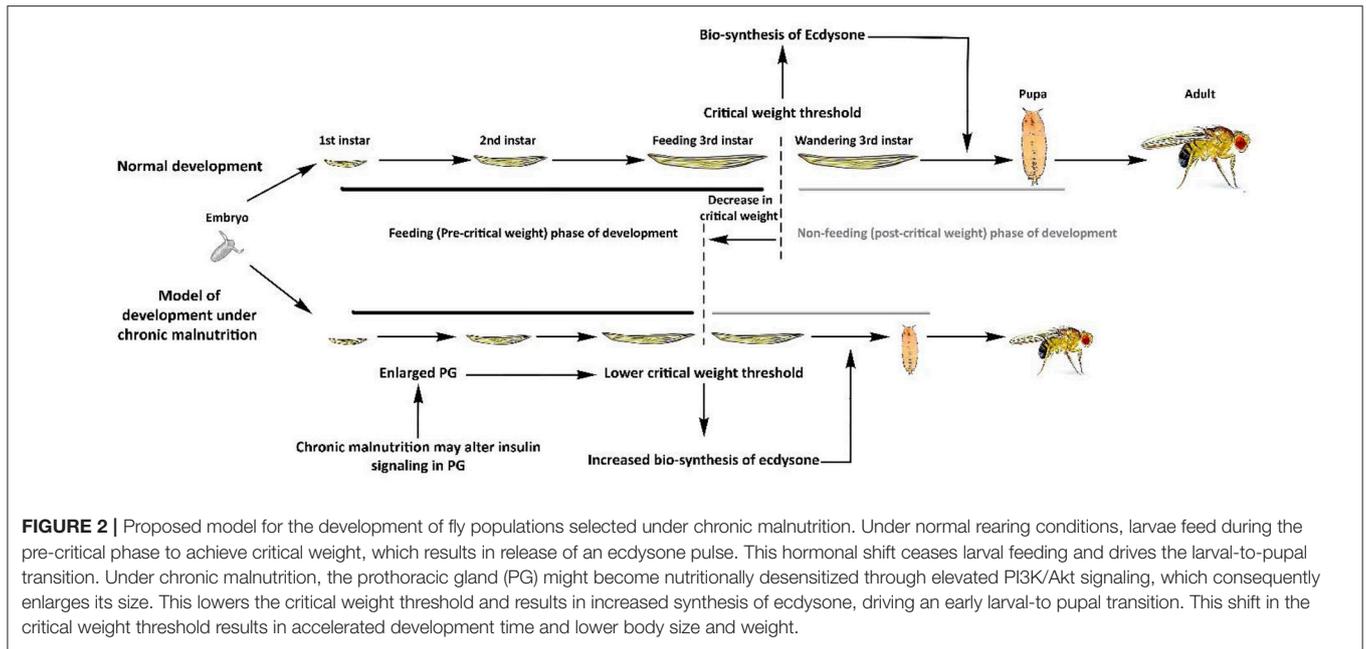


and body size of the fly. These observations might suggest that increased PG-mediated ecdysone signaling underlies the physiological phenotypes observed in fly populations under chronic malnutrition (Kolss et al., 2009; Vijendravarma et al., 2011, 2012a; **Figure 2**).

How might PG-mediated ecdysone signaling be enhanced under malnourished conditions, when PI3K/Akt signaling is typically reduced? Specific tissues differ in their sensitivity to nutrients and PI3K/Akt signaling (Shingleton et al., 2005) to result in organ-specific growth—a phenomenon known as allometry. Some organs, such as the male genitalia of the fly, do not reduce in size in response to nutritional stress; they are insensitive to the nutritional environment (Shingleton et al., 2005; Shingleton, 2010; Tang et al., 2011). This reduced sensitivity to nutrition is attained by maintaining high TOR signaling, or through tissue-specific reduction in the FOXO transcription factor under low nutritional conditions (Tang et al., 2011;

Koyama et al., 2013). This mechanism would allow organs to become less sensitive to nutrition in order to mollify the effects of poor nutrition. Hence, it is possible that, under chronic nutritional stress, the PG might be less nutritionally sensitive and maintain or increase PI3K/Akt signaling, resulting in a relatively enlarged PG. It will be of interest to test if the expression of PI3K/Akt signaling components is indeed upregulated under chronic nutritional stress.

Broad regulatory functions of Akt also include cell growth, control of apoptosis, and cell proliferation (Manning and Cantley, 2007). Hence, the PI3K/Akt pathway can directly impact cell size and number to potentially regulate body size (Goberdhan et al., 1999; Verdu et al., 1999; Zhang et al., 2000; Miron et al., 2001). Indeed, mutants of *chico* show reduced body size due to a reduction in both cell number and size (Böhni et al., 1999). In contrast, evolutionary changes in wing size under chronic malnutrition were mediated entirely by



reduced cell number (Vijendravarma et al., 2011). These studies might imply that pathway components upstream or at the level of Akt might be the targets of selection under nutritional stress.

FORAGING BEHAVIOR

In addition to physiological responses, foraging behavior is also influenced in *Drosophila* exposed to poor nutritional conditions (Sokolowski et al., 1997; Vijendravarma et al., 2012b). *Drosophila* larvae spend most of their time feeding and naturally exhibit a foraging polymorphism driven by the *foraging* (*for*) gene (Osborne et al., 1997). *for* encodes a cGMP-dependent protein kinase (PKG) (Osborne et al., 1997) and has been shown to alter larval nutrient acquisition in response to food deprivation (Kaun et al., 2007, 2008). In the presence of food, rovers (*for^R*) have a longer foraging path and a higher propensity to leave a food patch as compared to sitters (*for^S*). However, in the absence of food, these variants do not exhibit any difference in activity (Sokolowski et al., 1984; Kaun et al., 2007).

The evolution of *Drosophila* foraging behavior has been reported under density-dependent selection whereby high larval density reduces food access and induces nutritional stress on unsuccessful competitors (Ruiz-Dubreuil et al., 1996; Sokolowski et al., 1997). Interestingly, the rover phenotype was selected under high-density rearing (Sokolowski et al., 1997). In contrast, populations evolved under chronic larval malnutrition without crowding showed a shorter foraging path length compared to unselected controls (Vijendravarma et al., 2012b). Thus, the context of how malnutrition is applied plays a role in the evolution of behavior. In a competitive environment, greater mobility increases the likelihood of exploiting heterogeneous food or finding a better nutritional niche. However, the energetic

cost of locomotion in *Drosophila* larvae (Berrigan and Pepin, 1995) might not be favorable in a non-competitive context.

Similar to larvae, adult rovers and sitters show variation in their feeding behaviors. Rover adults exhibit a greater proboscis extension response to sucrose than sitters under food deprivation (Scheiner et al., 2004; Hughson et al., 2017). After sucrose feeding, rover and sitter adults exhibit different walking patterns—rovers travel further compared to sitters (Pereira and Sokolowski, 1993). In addition, rovers are more sensitive to starvation and show extended food search patterns following food deprivation (Hughson et al., 2017). Genomic and metabolomic analyses of rover and sitter adults demonstrated that the insulin-mediated PI3K/Akt pathway might be responsible for the rover- and sitter-dependent feeding behaviors under food deprivation (Kent et al., 2009). Compared to sitters, rovers show higher PI3K/Akt signaling (Kent et al., 2009). Combining *for* alleles with PI3K/Akt signaling mutants changed rover-like behavior into sitter-like behavior in transheterozygote animals, further suggesting an epistatic link between the PI3K/Akt and *for* pathways (Kent et al., 2009). This also indicates that PI3K/Akt signaling attenuation might lead to sitter-like behavior under nutrient deprivation, further supporting our speculation that PI3K/Akt pathway components might experience selective pressures under chronic malnutrition. Future work could help assess potential molecular interactions between the PKG and PI3K/Akt pathways.

LEARNING AND MEMORY

Selection under long-term malnutrition was also reported to affect the learning ability of *Drosophila* (Kolss and Kawecki, 2008). Flies reared on a high carbohydrate-low protein diet exhibit impaired visual learning and memory formation (Xia

et al., 1997). Conversely, fly populations selected for 51 generations on conditions that improve learning and memory (Mery and Kawecki, 2002) exhibited reduced larval competitive ability for a limited food source (Mery and Kawecki, 2003). This suggests a direct relationship between the availability of nutrients and learning and memory. However, the underlying mechanism of the effect of nutritional stress on memory formation is still unclear.

PI3K/Akt signaling has also been implicated in long-term memory formation (Chambers et al., 2015). Genetic impairment of this signaling cascade in *Drosophila* larval mushroom bodies, a pair of structures responsible for memory formation, hampers feeding behavior by causing a reduction in food intake (Zhao and Campos, 2012). Interestingly, the *for* rover/sitter polymorphism has also been shown to mediate associative learning and memory in *Drosophila* (Mery et al., 2007). *for^R* flies tend to have weaker long-term memory compared to that of *for^S* (Mery et al., 2007). Taken together, these studies further support the idea that there is a molecular link between the PI3K/Akt and PKG pathways (Kent et al., 2009) and that PI3K/Akt might be a possible selection target under chronic malnutrition.

CANNIBALISM

Drosophila melanogaster is an herbivorous and detritivorous species which primarily feeds on a rotten vegetative matter and associated microbes (Carson, 1971; Yamada et al., 2015). However, under nutritional stress, larvae can cannibalize and feed on conspecifics (Vijendravarma et al., 2013; Ahmad et al., 2015). Larvae selected on chronic malnutrition locate and prey upon conspecific victims more efficiently than non-selected controls, suggesting that cannibalism is an adaptive trait (Vijendravarma et al., 2013). Typically, *Drosophila* is morphologically ill-equipped to scavenge on a carnivorous diet. However, feeding exclusively on cannibalistic diet induces larval mouth hook plasticity by increasing the number of teeth (Vijendravarma et al., 2013). This allows larvae to puncture a victim's cuticle during cannibalism. The adaptive significance of this plastic phenotype, as well as its molecular basis, are still not known. Since cannibalistic behavior is reported to evolve under chronic malnutrition, it will be interesting to probe if there exists a link between the PI3K/Akt pathway and cannibalism.

CONCLUSION

Recent studies have exploited the advantages of using *Drosophila* to demonstrate evolutionary responses to chronic nutritional stress (Kolss and Kawecki, 2008; Kolss et al., 2009; Vijendravarma et al., 2011, 2012a,b, 2013, 2015; Zajitschek and Zajitschek, 2016). However, the genetic and molecular mechanisms underlying changes in physiology and behavior are still mostly unknown. By reviewing phenotypic studies of fly populations under chronic malnutrition, we speculate that components of the nutrient-sensing PI3K/Akt pathway might be among the selection targets under nutritional stress; a

number of physiological and behavioral traits that emerge under nutritional stress parallel those of PI3K/Akt pathway mutants. If the PI3K/Akt pathway is a target of selective pressure under chronic malnutrition, genetic signatures of selection and altered expression of its components could be revealed by genomic sequencing and expression profiling. Additionally, proteomic profiles of animals selected under chronic nutritional stress could be used to identify post-translational modifications that contribute to alterations of the PI3K/Akt pathway.

Next-generation sequencing technologies have greatly facilitated evolve-and-resequence studies, which attempt to identify genetic variants causative of evolved traits. Recently, resequenced *D. melanogaster* strains evolved under low oxygen levels showed signatures of selection acting on Notch pathway signaling; follow-up analyses on genetic mutants supported the idea that the Notch pathway is involved in hypoxic responses (Zhou et al., 2011). Furthermore, *D. melanogaster* strains sequenced after multi-generational exposure to the natural fly virus, *Drosophila* C virus (DCV) identified candidate DCV resistance genes that were subsequently verified in mutational analyses (Martins et al., 2014). While genome-wide approaches might uncover pathways that are under selective pressure, including or independent of PI3K/Akt signaling, there will still be challenges in identifying candidate genes involved in polygenic traits. In fact, many evolve-and-resequence studies using *D. melanogaster* have highlighted the difficulties in characterizing experimental evolutionary changes given the complexities revealed in associating phenotypic output with specific alterations within the genome-wide databases (Turner et al., 2011; Turner and Miller, 2012; Reed et al., 2014; Griffin et al., 2016). Thus, although genomic sequencing could facilitate future studies of the molecular basis of adaptation to nutritional stress, a more targeted approach informed by a comprehensive examination of relevant studies might be more fruitful.

A major technical issue to overcome is the lack of a defined standard medium, resulting in the use of different fly food recipes across labs (Marx, 2015). Varying different components of fly food can alter the carbohydrate-protein ratio (Bruce et al., 2013; Tatar et al., 2014; Lee, 2015). These changes might have large impacts on studies dealing with selection over multiple generations. Furthermore, altering the concentration of different nutrients can result in a range of responses in flies (Lee et al., 2008; Andersen et al., 2010; Sisodia et al., 2015) and preclude the identification of specific nutritional changes associated with adaptive responses. Future studies using chemically defined diets will improve the comparison of empirical findings across labs (Piper et al., 2014). A nutritional geometry approach (Simpson and Raubenheimer, 1999, 2012) might be needed to comprehensively quantify the specific contributions of macro- or micro-nutrients. Fortunately, the large number of diets needed for these types of studies are feasible using *Drosophila*. The classical components of the PI3K/Akt pathway are evolutionarily and functionally conserved in mammals (Oldham, 2011). Therefore, studies using the fly model may help us better understand

the molecular basis of development and evolution across species.

AUTHOR CONTRIBUTIONS

MA drafted the paper. All authors contributed equally to the conception and revision of the paper.

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ACKNOWLEDGMENTS

This work was supported by grant R01AG045036 from the National Institutes of Health (WJ) and the Higher Education Commission of Pakistan (HEC/2228 and HEC/5909, MT). EK is an O’Keeffe Neuroscience Scholar of the Esther B. O’Keeffe Charitable Foundation.

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Conflict of Interest Statement: 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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