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Research Article

Drosophila Species as Models for Nutritional Studies: Development, Metabolic Pools on Diets with Contrasting Relative Sugar: Protein Ratios

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Abstract

We examined development time, survival, adult body weights, and metabolic pools of protein, triglycerides and glycogen in three *Drosophila* species reared on three isocaloric diets differing in their relative ratios of sugar to protein. *Drosophila melanogaster*, a fruit breeder, survived and developed well on all three diets. But two other species, the cactophilic *D. arizonae* and *D. mojavensis*, normally accustomed to low carbohydrate resources in nature, were significantly impaired by diets higher in sugar. As expected, based upon their natural history, *D. arizonae* was less affected than *D. mojavensis*. These species, whose genomes are sequenced and have many homologues with human metabolism genes, provide an inexpensive and tractable model system to study human metabolic diseases related to excess dietary sugar.

Keywords: *Drosophila*; Nutrition; Diet; Metabolic pools; Development

Abbreviations: HPLS: High Protein-Low Sugar Diet; EPS: Equal Protein-Sugar Diet; LPHS: Low Protein-High Sugar Diet; MYA: Million Years Ago

Introduction

Metabolic diseases have reached epidemic proportions in developed and developing countries [1-4]. This increase in metabolic disease appears to be associated with the transition from traditional diets to diets high in sugars, fats and processed foods at the same time that physical activity has decreased [5,6]. Among the most obvious effects are the increase in obesity, diabetes and hyperlipidemias. But diet and

lifestyle clearly interact with individual genotypes to produce a wide range of phenotypic responses with respect to obesity and illness [5,7].

Drosophila melanogaster has become a highly effective tool in studies of metabolism and metabolic disorders, because many fly genes have human homologues or human disease cognates [8]. Testing various genotypic responses to dietary manipulation is

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more easily accomplished in flies than in other model organs. For example, 146 inbred isofemale lines of *D. melanogaster* reared on diets differing in fat and sugar, showed a significant genotype by diet interaction for traits such as triglycerides, glycogen, survival and weight [9-11]. Within *D. melanogaster*, these authors conclude that the way in which a dietary change affects an individual's phenotype reflects the individual's genetic make-up.

A multispecies comparative analysis provides an additional approach to genotype by environment interactions. Our laboratory [12] compared the survival and metabolism of *D. melanogaster*, a fruit breeder with *D. mojavensis*, a cactus breeder, on isocaloric diets that were; (1) high in protein and low in sugar (HPLS), (2) equal in protein and sugar (EPS), or (3) low in protein but high in sugar (LPHS). These two species exhibited profoundly different responses to the diets when reared on these diets from the first instar. *Drosophila melanogaster* showed only marginal decrements in survival or development time in diets high in sugar relative to diets where more of the calories came from protein.

Drosophila mojavensis, on the other hand could not develop or survive on the LPHS food and had greatly reduced survival on the EPS food. Adults of both species showed increasing triglyceride levels with increasing sugar in their larval food. Although no *D. mojavensis* survived in the LPHS diet, those surviving in the EPS food had nearly twice the triglyceride levels as those having developed in HPLS. Flies from both species showed increases in glycogen as the amount of sugar increased, but the levels of glycogen in *D. mojavensis* were significantly less than in *D. melanogaster* [12]. These observations suggest that different *Drosophila* species may provide an inexpensive model system for studies of nutrition and metabolism.

How can these species differences be explained? Ecologically speaking, *D. melanogaster* breeds in fruits, where the sugar content is high compared to *D. mojavensis*, which diverged approximately 40MYA from *D. melanogaster* and is strictly cactophilic. Its cactus hosts are low in carbohydrates [13]. Their low survival and perturbed metabolism in the face of even slightly

more sugar therefore is not surprising. If the difference between these two species are a reflection of their evolution and natural history, species closely related to *D. mojavensis* but that consume more sugars in nature should exhibit fewer problems with the laboratory diets higher in sugar. In fact, *D. mojavensis* has a close relative, *D. arizonae*, which, although it breeds in cactus in much of its range, also breeds in fruits making it more of a dietary generalist. We reasoned that if *D. arizonae* is indeed more accustomed to more sugars, than *D. mojavensis*, it should have relatively higher survival to adulthood on all of the three diets than its close relative.

If this is the case, the two species could lend themselves to additional genetic and metabolic studies since they are able to hybridize. We tested this prediction here and found that while *D. arizonae* does have impaired survival with increasing sugar levels relative to *D. melanogaster*, it indeed outperforms *D. mojavensis*, while exhibiting increases in triglycerides and glycogen in the highest sugar diet.

Materials and Methods

Flies

Multi-female strains of each of the three species were used in examining the effect of diet on development times and survival to adulthood. The *D. melanogaster* strain had been collected in San Diego, California (USA) in 2008 (TAM); *D. arizonae* was from La Paz, Baja California Sur (Mexico) in 2013 (TAM) and *D. mojavensis* from Organ Pipe National Monument Arizona (USA) in 2008 (TAM). Strains of each species were maintained in multiple vials, mixing them every generation to maintain adequate genetic variability. All stocks, prior to use in experiments, were grown at room temperature (~25°C) in standard banana-opuntia media. Sexually mature adults were placed in yeasted egg collecting chambers (Genesee Scientific) and the embryos were allowed to develop to early second instar at which time they were transferred to the experimental diets (below). The early second instar larvae first were washed with distilled water, to remove traces of yeast and transferred with a camelhair brush to experimental food vials (n=30 flies/vial, five replicates/diet). Vials were checked daily for the (1) timing of pupation and (2)

timing adult emergence as well (3) proportion surviving to adulthood in each diet.

Experimental diets

Iso caloric artificial diets were prepared as follows: a diet with high ratio of protein:sugar or HPLS a diet with equal protein:sugar or EPS, and a diet with low ratio of protein:sugar or LPHS [12]. Protein was supplied by active dry yeast and sugar by sucrose, complemented with yellow cornmeal and agar. All ingredients were mixed and boiled, with methyl-paraben added as a fungal inhibitor.

Statistical analyses

We utilized JMP v 13 (SAS Institute, NC) to perform ANOVAs of survival, development time, weights and metabolic pools. Survival percentages were arc sin transformed prior to analysis.

Results

Survival

The delay in development time was paralleled by the decrease in survival. Survival of second instar larvae to adulthood of the three species on each diet is shown in Figure 1b. Not even *D. melanogaster* had 100% survival in the high protein food, probably owing to unavoidable injury to larvae during transfer, but diet and species had significant effects on survival (Supplementary Table 1a). The main effects Diet and Species were significant and to a lesser degree, the interactions Diet x Species and Species x Sex. While the survival of *D. arizonae* was also severely impacted by the increasing sugar relative to protein, more survived than did *D. mojavensis*.

Development time

All three species showed delayed pupation as sugar increased and protein decreased (Figure 1a). Significant terms in the ANOVA were Diet, Species and Species x Diet (Supplementary Table 1b). Delayed pupation was most severe in *D. mojavensis*. Pupation time in *D. melanogaster* was the least affected by the diets while *D. arizonae* pupated faster than its sister species, *D. mojavensis*. Once having reached pupation, species differed in the time they required to undergo

metamorphosis and eclose as adults (Figure 1a). Diet and species as well as their interaction were significant factors in the delays (Supplementary Table 1c).

Dry weights

Dry weights for flies reared on the different diets are presented in Figure 2a. Owing to the lack of survival of *D. mojavensis* in the LPHS diet, no weights were available under that condition for this species. In comparing *D. arizonae* and *D. melanogaster* reared on the three diets, all main effects were highly significant as were interactions Diet x Species and Diet x Sex (Figure 2a). Weights of all three species could be compared for the HPLS and EPS diets and all main effects were significant (Supplementary Table 1d).

A general pattern seen in the data is that whether statistically significant or not, flies of all three species appear to attain the greatest weights on the EPS diet.

Metabolic pools

ANOVA of comparisons in all metabolic pools could only be performed for the three species on the HPLS and EPS diets owing to the lack of sufficient material for *D. mojavensis* on the LPHS diet. Protein pools of the bodies of all three species tended to decline with reductions in the relative levels of protein in their larval diets (Figure 2b). The overall model was highly significant with major effects Diet and Species being highly significant as well as the Diet x Sex and three-way interaction terms (Supplementary Table 1e).

While protein pools declined with more relative calories coming from sugar, triglycerides tended to increase in all three species (Figure 2c). Again the ANOVA could only be performed for the two species on the three diets, but it was highly significant with the main effect of diet being highly significant as were the Diet x Species, Diet x Sex, and Species x Sex interaction terms (Supplementary Table 1f).

Pools of glycogen also increased in flies having been reared in the diets with relatively more sugar (Figure 2d). The EPS diet produced an especially large spike in the glycogen pool of *D. arizonae*. The highly significant ANOVA on the two species *D. melanogaster* and *D.*

Figure 1: (a) Developmental times and survival of *D. melanogaster*, *D. arizonae*, and *D. mojavensis* reared on three different diets: Time from second instar until pupation in black bars and from second instar until adult emergence on the three isocaloric diets in white bars. (b) Mean survival (+ SE for five vials/treatment) of *D. melanogaster*, *D. arizonae*, and *D. mojavensis* as measured by proportion of second instar larvae producing adults, when reared on the three different isocaloric diets.

Black bars = females, white bars = males.

arizonae revealed a large impact of the effects Diet, Species, Diet x Sex, Species x Sex and the three-way interaction terms (Supplementary Table 1g).

Discussion

As we predicted based upon the ecology and natural history of the three species, *D. melanogaster* had the best survival and *D. mojavensis* the worst when larvae experienced relatively higher proportions of sugar relative to protein in their diets. The sister species to *D. mojavensis*, *D. arizonae*, which is more of a dietary generalist and often associates with commercial fruits, as we predicted, had somewhat higher survival on diets higher in sugar. The higher survival allowed us to examine other phenotypes such as development time,

weight and metabolic pools.

In all cases except *D. melanogaster* females, dry weight increased in flies reared on EPS compared to HPLS diets. The impact of increasing sugar in the diet parallels what has been reported in humans, where maternal sugar consumption leads to higher birth weight babies [14,15]. Our highest sugar diets are not associated with even higher weights, however, which may reflect the reduction in protein, as development times are also prolonged in the LPHS diets. Thus while tempting to attribute the decrements in survival and development time only to high sugar, the reduced protein or minor difference in mineral content also may have been factors.

Figure 2: (a) Dry weights, metabolic pools of (b) protein, (c) triglycerides and (d) glycogen in three *Drosophila* species reared on three isocaloric diets.

The fact that these three species respond differently to the three diets points to genetic variation in the underpinnings of how developmental nutrition affects growth and metabolism. Most human metabolic disease genes have homologues in *Drosophila* [8], making these flies a very attractive model for empirical nutritional studies, especially given that the genomes of all three already are sequenced [16,17]. Indeed, just like in humans, fruit flies have receptors devoted to sweet and bitter tastes. The major difference is that mammalian taste receptors are expressed only on the tongue, while fly taste receptors are located on many structures including the proboscis, feet, and wings [18,19]. Variants in the sequences of the taste receptors in these three species may be detectable in the sequenced genomes. The three species also fit well with Neel's concept of the "thrifty genotype", in which some human populations, because they often faced lean times, are adapted to store nutrients when available [20]. Thus an excess of calories, especially combined with

a less physically active life style, leads to obesity and metabolic diseases such as diabetes.

Drosophila melanogaster can be thought of as well adapted to a "Western Diet", and while *D. mojavensis* is not, *D. arizonae* represents more of an intermediate. Thus *D. arizonae* and to a greater extent, *D. mojavensis*, can be thought of as having the "thrifty genotype" while *D. melanogaster* does not. The difference is most striking in the glycogen pools of *D. arizonae* reared on diets high in sugar. Examining the transcriptomic responses of these three species to the different diets will help identify particular genes that respond to the diets. Are particular genes up or down regulated in *D. arizonae* or *D. mojavensis* in the presence of high sugar and low protein in comparison to *D. melanogaster*? If so, what metabolic pathways are involved? The flies also can be utilized to examine additional phenotypic effects of these diets, such as impairment of longevity, normal behaviors and reproductive fitness.

Subsequent studies also should evaluate the fitness and metabolic pools of hybrids between *D. arizonae* and *D. mojavensis* to determine the genetic basis and architecture of the differences between them in their response to the different diets. Because they can produce viable hybrids these two species represent an unprecedented and inexpensive model system for genetic studies metabolic and development responses to various diets.

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Author Contributions

NNY, MRLL and TAM designed the experiments, NNY performed the experiments, MRLL assisted with media preparation, analyses and figure preparation, NNY and TAM wrote the manuscript.

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