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Evolution of starvation resistance in *Drosophila melanogaster*: Measurement of direct and correlated responses to artificial selection

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Abstract

Laboratory selection for resistance to starvation has been conducted under relatively controlled conditions to investigate direct and correlated responses to artificial selection. With regard to starvation resistance, there are three physiological routes by which the trait can evolve: resource accumulation, energy conservation and starvation tolerance. A majority of energetic compounds and macromolecules including triglycerides, trehalose and other sugars, and soluble protein increased in abundance as a result of selection. Movement was additionally investigated with selected males moving less than control males and selected females exhibiting a similar response to selection. Results obtained from this study supported two of the possible evolutionary mechanisms for adaptation to starvation: energy compound storage and conservation. If the response to selection is based on an evolutionarily conserved pattern of genetic correlations (elevated lipid, elevated sugars and reduced movement), then the response to selection is medically relevant and the genetic architecture should be investigated in depth.

Keywords: body composition, Drosophila, laboratory selection, movement, starvation

Introduction

In natural populations, reductions in available food resources are common. The physiology of adaptation to starvation is a subject of general interest for the study of evolution and its potential impacts on human health. A comparative study of starvation and desiccation survival in *Drosophila melanogaster* analyzed patterns of variation between species, between populations, and within populations (Hoffmann & Harshman, 1999). In general, starvation resistance is correlated with relatively long lifespan, slow development, reduced egg production and large body size. These correlations are based on phenotypic data, but it is reasonable to hypothesize that these associations are evolutionarily derived and have a genetic basis. Experimental evolution of starvation resistance in the laboratory can yield insight into the mechanism of evolution within the laboratory (Rion & Kawecki, 2007) and in natural populations.

A series of laboratory selection experiments for starvation resistance have been conducted using *D. melanogaster* with acute starvation as the selective agent. Six sets of lines have been directly selected for starvation resistance with some maintained at a selection intensity of approximately 50% mortality each generation similar to the selection pressures used within this study. There has been a significant and often substantial direct response to selection in all six sets, indicating widespread genetic variation for this trait. The correlated (indirect) responses to selection are of particular interest as they can provide insight into mechanisms underlying the response to selection. The first laboratory evolution study of starvation resistance was based on a set of lines selected at the University of California at Irvine (Rose *et al.*, 1992). These lines were investigated based on correlated responses to selection: life history, stress survival, phenotypic plasticity and physiology. Within this study, selection for starvation resistance was correlated with increased lifespan. Additional laboratory selection

experiments for starvation resistance were based on two separate selection experiments (Harshman & Schmid, 1998; Harshman *et al.*, 1999a,b). These lines were investigated in terms of life history changes, stress responses, phenotypic plasticity, physiology and enzyme activities. Selection for starvation resistance altered body composition (increased triglycerides) and enzymes associated with lipid biosynthesis. Per fly respiration rate did no decrease as an indirect response to selection. More recent starvation resistance selection experiments included three further sets of starvation-selected lines (Bubliy & Loeschcke, 2005; Hoffmann *et al.*, 2005; Baldal *et al.*, 2006). In two of these studies, the evolution of correlated stress responses was the primary research focus (Bubliy & Loeschcke, 2005; Hoffmann *et al.*, 2005). Bubliy & Loeschcke (2005) found that lines selected for starvation resistance had an increased development time, but no significant correlation with heat-shock or cold shock resistance, heat knockdown resistance or desiccation resistance. Hoffmann *et al.* (2005) indicated a sex-specific trade-off between starvation resistance and cold resistance. In the remaining selection experiment, a range of traits were measured as correlated responses to selection: metabolic rate, oxidative stress survival and lifespan (Baldal *et al.*, 2006). Baldal *et al.* (2006) found that fat content increased in the starvation-selected lines and that there was no difference in metabolic rate between selected and control lines. However, there was an interaction between starvation and line type such that the starved selected flies had the highest metabolic rate.

Correlated responses to selection for starvation resistance allow for investigation into the mechanisms underlying the response to selection and the relationship of natural populations to laboratory-based studies. Insight into the mechanisms underlying selection is of particular interest. In the case of starvation resistance, there are three physiological routes by which traits can evolve: resource accumulation, energy conservation and starvation tolerance (Rion & Kawecki, 2007). Starvation-selected lines are well suited for the investigation of each general mechanism in terms of correlated responses to selection. The information generated from addressing these questions of the evolution of starvation resistance can be used to provide insight into adaptation to food deprivation in natural populations as well as investigate human health implications attributed to excess nutrient storage.

This study is a relatively controlled laboratory selection experiment that measures direct and correlated responses to selection for starvation resistance. This study is necessary in that there have been no previous studies on the effect of selection for starvation resistance on abundance of a range of energy-containing compounds and macromolecules nor on movement as a potential mechanism for energy conservation. Also, the effect of starvation itself has been considered throughout the study. The level of selection on females and males was approximately 50% mortality during each generation for 15 generations. The number of breeders was held constant for each population, which increased consistency within the selection experiment. The direct response to selection and all correlated responses were measured on *D. melanogaster* derived from selection generation 15. A range of body composition measurements were obtained for both starved and unstarved adult flies of both sexes taken from the selected and control lines. This included soluble protein, glycogen, total sugar, trehalose and triglycerides. Using physical methods, the proportion of triacylglycerides (TAGs), diacylglycerides (DAGs) and free fatty acids (FFAs) was estimated. Movement of flies from the selected and control lines was additionally measured. The breadth of body composition measurements was obtained, and the inclusion of movement is a unique feature of this study. The lean mass proportion of energetic compounds increased in the selected lines relative to the control lines and decreased as a function of starvation. Movement was reduced in selected line males as a correlated response to selection, with a corresponding trend observed in females.

Materials and methods

The basic experimental design in this study was to use eight outbreeding subpopulations ('lines' always refers to one or more of the subpopulations) derived from one base population. Four of the lines were selected for starvation resistance in a relatively precise manner, and the other four lines were unselected controls. All the lines (selected and control) were used for all experiments reported in this study. Flies were selected for starvation resistance by placing a large number of one sex in boxes (cages) with ample access to water in a high humidity environment, but no explicit source of food was present. After generation of selection, all the lines were investigated in terms of two kinds of measures: body composition and movement.

Establishment of the populations used for selection and the method of selection for starvation resistance

The base population used for selection was derived from inbred lines produced from mated females collected at the Wolfskill Experimental Orchard in Yolo County, California. Following collection, the females were brought into the laboratory to establish isofemale lines. From the inbred lines, 10 were chosen at random to establish an initial population for selection. The base population was established by intercrossing all the inbred lines in all possible combinations, including reciprocal crosses. Approximately 15,000 flies were used to establish the initial base population, which remained at an average population size of at least 10,000 flies for 2 years prior to beginning the present selection

experiment. The population was maintained using an overlapping generation population regime in which 20 bottles were present in the cage, and each week the four oldest bottles were replaced. An overlapping population regime has been shown to contribute to the maintenance of relatively long lifespan and increased stress resistance compared to a batch culture procedure for population maintenance in the laboratory (Hoffmann *et al.*, 2001; Linnen *et al.*, 2001).

Prior to initiation of selection, the base population was divided into eight subpopulations, which were used to produce four replicate lines for selection and four replicate control lines. Each subpopulation was maintained at 25°C and 12:12 L:D at a population size of approximately 4000 randomly mating adults that were derived from vials seeded with a constant number of eggs (100 eggs per vial). This discreet population regime was continued for four generations prior to conducting selection.

Selection

Selection for starvation resistance was conducted on adult mated males and females that were approximately 7 days old upon initiation of selection. For this purpose, 2000 males and 2000 females were obtained from each of the eight subpopulations to establish four selected and four control lines. Adult flies of one sex from each line were placed in population cages at the start of selection. Males and females were placed in separate cages partially because males die at a faster rate than females and it would be difficult to control the level of mortality for both sexes if they were mixed within a cage.

In the cages, males and females within the control lines were provided with six Petri plates containing food, whereas flies in the replicate selected lines received six plates containing solidified agar as a water source. Plates were changed every other day during the morning time-point to provide flies with adequate food or water. Relative humidity within the cages was maintained by placing the cages within a clear plastic bag containing a damp paper towel. The moistened paper towel was replaced in conjunction with removal and replacement of food or agar plates.

During the process of selection for starvation resistance, the response to selection was assessed in each individual cage by tabulating mortality levels at 12 h intervals, at which time dead flies were removed by aspiration. Upon reaching 50% mortality in the selected lines, flies were removed from the selected line cages containing solidified agar and control line flies were removed from cages with food. Flies were removed from the cages by aspiration following brief exposure to carbon dioxide. *Drosophila melanogaster* from each line were placed in plastic bottles with fly medium at a density of approximately 150 flies per bottle. The flies were allowed a 2-day recovery period prior to breeding to produce the next generation. A standard number of 75 males and 75 females in six bottles from each of the replicate selected and control lines were allowed to mate. The bottles were used to harvest eggs, and a standard number of eggs were collected per vial. From these eggs, a population of 2000 males and 2000 females for each individual replicate line were obtained for each generation of selection.

To determine the direct response to selection, flies from selected and control lines were placed in cages containing Petri plates with only solidified agar. The relative humidity was maintained within the cage by wet paper towels placed in the surrounding clear plastic bags. Using flies two generations removed from selection, the time to total mortality was determined for each line. Mortality levels were tabulated at 12-h intervals until all the flies were dead. Movement, body composition and weight measurements were obtained from flies that were 3–6 generations removed from selection (relaxed selection).

Movement

The number of movements of individual *D. melanogaster* from the selected and control lines following either 32 h of starvation or 32 h in the presence of food was determined using individuals that were six generations removed from 15 generations of artificial selection for starvation resistance. Mated flies selected for movement analysis were exposed to the presence or absence of starvation at 7–8 days post-eclosion prior to placement within glass capillary tubes (5 diameter by 65 mm length). Once inside the tubes, the flies were allowed to recover from ethyl ether exposure used to separate the sexes prior to experimentation. Food was provided at both ends of the capillary tube, with one end only partially covered to allow airflow.

The placement of each individual within the 64 total spaces between the two *Drosophila* activity monitors (TriKinetics, Waltham, MA, USA) was determined using a statistical randomization scheme generated by SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Implementation of statistical randomization of sample placement within each of the monitors was used to reduce or eliminate positional effects in the acquisition of data. Each capillary tube was centred with respect to the monitor and secured in place with a rubber band.

Detection of the number of movements of individual flies was accomplished using an infrared beam that bisected the glass capillary tube located within each position of the monitors. Each time an individual fly crossed the beam, the computer recorded the movement. The number of movements was quantified for each of the six total replicates obtained from each selected and control line exposed to the presence or absence of starvation. Movement was recorded in 10-min intervals for 48 h. This allowed for the identification of variation within

and among lines and treatments with respect to alterations in the light cycle. Environmental conditions were held constant during experimentation at 25°C with a 12:12 L:D cycle. Relative humidity was maintained using moistened cotton balls placed within clear plastic bags surrounding each monitor. The cotton balls were moistened with distilled water daily to prevent desiccation of the experimental subjects.

Body composition assays

Flies used in the body composition assays were allowed to mate prior to flash freezing the samples for extraction. Mated *D. melanogaster* used in body composition analysis were approximately 5–9 days old at the time of collection. Each sample of flies were homogenized in an Eppendorf tube using a 5 / 32-inch stainless steel grinding ball from OPS Diagnostics in a Talboys High Throughput Homogenizer (OPS Diagnostics, Sunnyvale, CA, USA) at 1120 oscillations for three minutes. Ten flies were homogenized for each of the three biological replicates of each sample. Following chemical treatment of homogenates for each assay, the reactions were read in a microtitre plate reader (VersaMax; Molecular Dynamics). For the assays described by Van Handel (1985), the optical density of the standards and samples was read at two wavelengths to increase the linearity of the readings.

Protein

Total soluble protein was determined using the Pierce BCA Protein Assay (Rockford, Illinois, USA), which is a copper-based assay that is relatively resistant to interference from nonproteinaceous compounds in solution. The microtitre plate protocol was employed.

Glycogen and total sugar

Quantification of glycogen and total sugar was performed using methods described by Van Handel (1985). After processing the homogenate, the supernatant containing the sugars was decanted and evaporated down. Glycogen obtained from the sample remained with the fly tissue after precipitation with sodium sulfate. Anthrone reagent was added to both the sugars and the glycogen. Optical densities were determined in a spectrophotometer at wavelengths of 555 and 625 nm.

Trehalose

Trehalose, a common disaccharide in insects, was quantified using methodology described by Van Handel (1985). Homogenates were heated at 90°C for 7 mins, which resulted in hydrolysis of sucrose to glucose and fructose while leaving trehalose intact. Addition of sodium hydroxide heated at 90°C led to the destruction of anthrone reactivity to glucose and fructose, allowing for the quantification of trehalose. Optical densities were measured in a spectrophotometer at wavelengths of 555 and 625 nm.

Triglycerides

The triglyceride abundance was determined using the BioVision Triglyceride kit (Mountain View, CA, USA). Lipase was added to homogenates, resulting in the cleavage of triglycerides to FFAs and glycerol for quantification. The optical density was read at 570 nm.

Dry weight and lean mass

Dry weight and lean mass measurements were obtained. Flies that were flash frozen in liquid nitrogen from each of the replicate selected and control lines (males and females, starved and unstarved) were used for weight determination. Flies were dried by adding 10 flies to an open 1.5-mL microfuge tube placed in a 65°C drying oven overnight. Each of the 10 flies was weighed using a Sartorius microbalance, and the average dry weight was obtained. Lean masses were determined using flies subjected to a Bligh & Dyer (1959) lipid extraction conducted prior to drying the samples overnight. Standardization of the body composition measurements was conducted using lean masses.

Estimates of lipid class proportions

Estimates of the proportion of lipid classes (polar lipids, TAGs, DAGs and FFAs) were conducted at the Kansas Lipidomics Research Center (KLRC) at Kansas State University on lipid samples extracted at the University of Nebraska-Lincoln. Each extraction contained 10 mated flies of the same sex at 5–9 days post-eclosion. *Drosophila melanogaster* were subject to a Bligh & Dyer (1959) lipid extraction and were then dried under a gentle stream of nitrogen in 2-mL glass tubes with Teflon caps prior to shipping to the KLRC. Samples and standards used routinely for the *Drosophila* were introduced into a tandem mass spectrometer by continuous infusion in solvent into the electrospray ionization source. The ion fragments of the lipids were separated in an electric field and sequentially scanned to identify lipids by class with peaks within individual lipid classes corresponding to different lipid species. Quantification of each lipid species occurred by comparison to internal standards.

The KLRC estimated the ratios of TAGs to polar lipids, DAGs to polar lipids and FFAs to polar lipids. For example, the normalized signal for total TAGs (nmol) was divided by the total nmol for polar lipids. This parameter was not an exact measurement of concentration, as the estimate of TAGs was not precise due to an inability to determine each of the three fatty acids present on each triglyceride molecule. The estimated ratios were not used for statistical analyses.

Statistical analyses

A mixed model analysis was conducted using SAS 9.2 software to analyze body composition, weight and movement. Lines were used as a random factor nested within the selection and environmental treatments. The

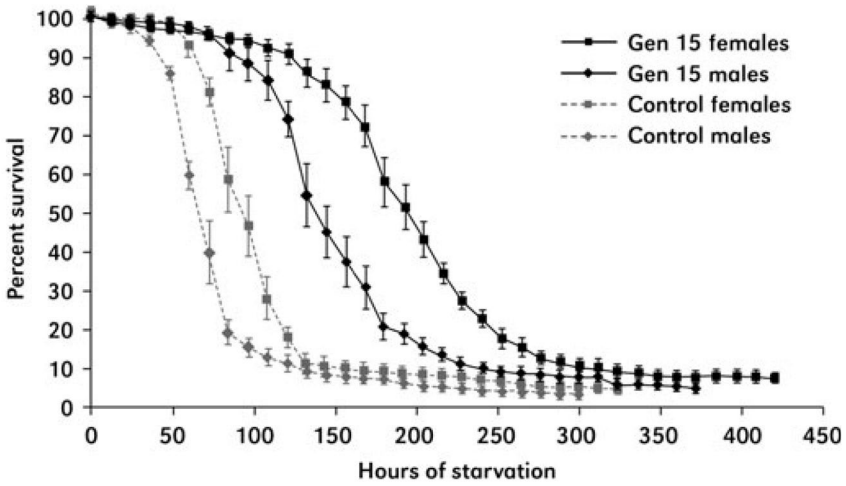

Figure 1. Acute starvation survival of females and males from selected and control lines after 15 generations of selection.

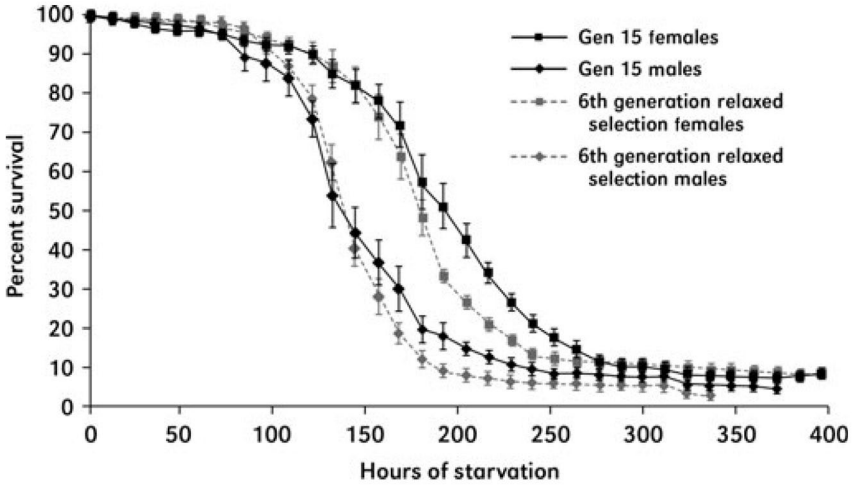

Figure 2. Acute starvation survival six generations after relaxation of selection following selection generation 15.

standard statistical comparison used in the study was to compare four selected lines and four control lines and thus there were six d.f. The means and variances compared were derived from the means of each of the selected or control lines.

Results

Responses to selection and selection relaxation

Figure 1 illustrates the mortality curves of flies held in cages with solidified agar with an external source of water vapor to maintain relative humidity. After 15 generations of selection, the selected and control lines were highly differentiated in terms of their survival curves (Fig. 1). There was a greater survival difference between selected and control line females than males. Selected lines had an extended period (approximate 100 h) of very low levels of mortality. Thereafter, the selected line flies had a shallower mortality curve than control line flies, which was especially pronounced in females. Figure 2 displays the mortality curves after six generations of relaxed selection following selection generation 15. This assay was conducted to determine whether starvation survival was lost as a function of relaxation while assays were being conducted. In males, the loss of starvation resistance is present, but the change is smaller than in females.

Body composition

Table 1 presents the mean values (SE) of body composition for selected and control lines under starved and unstarved conditions. All means are expressed in μg per mg of lean mass. In males, the level of soluble protein was not altered by selection (P = 0.4965), nor was it in females (P = 0.1655). There was no significant sex-by-treatment interaction for protein measurements obtained from either sex (female P = 0.6070, male P = 0.5891). For females, the effect of selection on glycogen

Table 1. Table values are means (SE) for line types (selected and control) under treatments (starved and unstarved) expressed as μg mg^{-1} lean mass. PRO, protein; GLY, glycogen; TS, total sugar; TRE, trehalose; TAG, triacylglyceride; F, female; M, male.

	Control		Selected	
	Unstarved	Starved	Unstarved	Starved
(F) PRO	795.1 (58.2)	787.7 (57.82)	764.8 (28.14)	724.3 (5.97)
(M) PRO	988.3 (42.1)	1199.9 (17.51)	1021.2 (60.21)	1108 (27.70)
(F) GLY	38.4 (1.94)	3.5 (0.53)	41.9 (5.58)	6.5 (1.18)
(M) GLY	28.7 (5.55)	2.2 (0.78)	34.1 (7.63)	7.5 (0.57)
(F) TS	21.3 (3.46)	9.2 (0.80)	29.0 (3.87)	14.5 (1.56)
(M) TS	27.0 (0.19)	8.4 (1.46)	28.2 (4.40)	18.6 (0.94)
(F) TRE	10.3 (1.02)	3.2 (0.80)	12.0 (2.07)	5.7 (0.43)
(M) TRE	6.4 (0.44)	0.2 (1.21)	7.6 (0.54)	2.8 (0.97)
(F) TAG	85.4 (7.64)	13.0 (2.90)	139.8 (23.30)	24.9 (5.72)
(M) TAG	28.7 (2.76)	6.5 (1.60)	66.5 (12.40)	15.6 (0.56)

was not statistically significant (P = 0.2686), whereas the effect of starvation was substantial (12.4% remaining, P < 0.0001). The same was observed for males: selection (P = 0.2686), starvation (15.4% remaining, P = 0.0002). In neither females nor males was there a significant sex-by-treatment interaction for glycogen abundance (female P = 0.9359, male P = 0.9922). Total sugars in females were significantly increased by selection (1.4-fold increase, P = 0.0291) and significantly decreased by starvation (47.1% remaining, P = 0.0005). In males, there was a statistically significant increase in total sugars by selection (1.3-fold increase, P = 0.0234) and a significant total sugar decrease after starvation (48.8% remaining, P < 0.0001). There was no statistically significant selection by starvation interactions for total sugars in females (P = 0.9359) or males (P = 0.6262) In general, selection increased total sugars and starvation decreased these sugars by approximately the same amount in females and males. For trehalose in females, selection had no significant effect (P = 0.1615), but starvation significantly decreased the level of this disaccharide (39.9% remaining, P = 0.0269). In males, selection resulted in a significant increase in trehalose (1.59-fold increase, P = 0.0269) and starvation resulted in a significant decrease (21.8% remaining, P < 0.0001). In neither females (P = 0.7981) nor males (P = 0.3946) was there a significant selection by starvation interaction. Generally, there was a trend for selection to increase trehalose, but this was statistically significant only in males, and in both sexes starvation reduced trehalose. For triglycerides measured in females, there was a significant increase following selection (1.68-fold increase, P = 0.0234) and a significant decrease after starvation (16.8% remaining, P < 0.0001). There was no significant selection by starvation interaction for triglycerides in females (P = 0.1152). In males, triglycerides increased markedly as a result of selection (2.33-fold, P = 0.0037) and decreased significantly after starvation (23.2% remaining, P = 0.0002). There was a statistically significant interaction between selection and starvation in males (P = 00422). Overall, selection increased the lean mass concentration of triglycerides and starvation decreased the level of triglycerides markedly in both sexes.

Dry weight and lean mass

Table 2 presents the data for dry weight and lean mass measurements. In both sexes, there was a statistically significant increase in dry weight (female P = 0.0090, male P = 0.0240) and lean mass (female P = 0.0280, male P = 0.0100) in response to selection and a decrease in dry weight (female P < 0.001, male P < 0.001) and lean mass (female P = 0.0280, male P < 0.0001) following starvation. There were no interactions between selection and treatment (dry weight: female P = 0.3120, male P = 01460; lean mass: female P = 0.5750, P 0.4630).

Estimates of the proportion of lipids

Table 3 presents estimates of the proportion of neutral lipids (TAGs, DAGs and FFAs) in relationship to the amount of polar lipids (phospholipids). The ratio is the mean (SE) normalized neutral lipid signal to the normalized polar lipid signal. For triglycerides, in the unstarved selected lines vs. unstarved control lines there is 2.1-fold increase (males) to 1.8-fold increase (females) in the level of triglyceride to polar lipids. Starvation

Table 2. Variates are means (SE) for line types (selected and control) under treatments (starved and unstarved) expressed as μg. F, female; M, male.

	Control		Selected	
	Unstarved	Starved	Unstarved	Starved
(F) Dry weight	461.8 (13.00)	359.8 (13.50)	533.5 (8.67)	411.0 (7.62)
(M) Dry weight	293.0 (6.98)	210.8 (4.72)	336.5 (3.86)	249.0 (5.18)
(F) Lean mass	321.0 (4.49)	298.8 (9.66)	349.0 (7.44)	325.3 (6.20)
(M) Lean mass	213.8 (3.35)	173.0 (4.10)	231.3 (4.72)	195.5 (3.80)

Table 3. Lipid class ratios to the amount of polar lipids were estimated by the Kansas Lipidomics Research Center using mass spectroscopy evaluation of lipid peak responses and internal standards. PL, polar lipid; TAG, triacylglyceride; DAG, diacylglyceride; FFA, free fatty acids.

	Control		Selected	
	Unstarved	Starved	Unstarved	Starved
(F) TAG/PL	2.8 (0.14)	1.6 (0.07)	5.1 (0.58)	3.1 (0.79)
(M) TAG/PL	1.8 (0.15)	0.89 (0.02)	3.8 (0.69)	2.5 (0.61)
(F) DAG/PL	0.86 (0.14)	0.36 (0.05)	0.77 (0.18)	0.44 (0.10)
(M) DAG/PL	0.39 (0.49)	0.24 (0.03)	0.53 (0.13)	0.39 (0.07)
(F) FFA/PL	0.31 (0.02)	0.30 (0.04)	0.26 (0.02)	0.25 (0.03)
(M) FFA/PL	0.39 (0.06)	0.32 (0.05)	0.29 (0.04)	0.36 (0.08)

decreased the TAG ratio approximately 39% (females) to 34% (males) in the selected lines and approximately 43% (females) to 50% (males) in the control lines. For DAGs, selection did not increase the ratio in selected females compared to control lines, but in males the ratio of DAGs to polar lipids increased approximately 36% as a result of selection. Starvation decreased the DAG level to a greater degree in the control lines than in the selected lines, which was additionally observed for the triglyceride ratio. Selection does not increase the FFA ratios in the selected lines as compared to the control lines. The FFA to polar lipid ratio tends to decrease after starvation, but the effect was not substantial and was not observed in selected males.

Movement

Table 4 indicates the average movement for males from selected or control lines, the effect of starvation, the effect of light, and the level of interaction between main effects. There was a statistically significant effect of selection on males ($P = 0.0006$), which was to move less. No significant effect of starvation was recorded ($P = 0.2351$), but the trend was for starvation to increase movement. There was an effect of light such that males moved more when the lights were off ($P < 0.0001$). A statistically significant interaction ($P = 0.0067$) between light and selection was detected and this was due to a greater increase in movement in the control lines as compared to the selected lines when the lights were on. Overall, for males, selected line flies moved less than the controls, starvation tended to increase movement and the effect of light was to reduce movement, which was most pronounced in the control lines.

Table 5 reports female movement data. In females, there was no statistically significant effect of selection for starvation resistance on movement ($P = 0.4018$), but the trend was reduced movement in the selected lines. There was no significant effect of starvation ($P = 0.3252$), but the trend was for reduced female movement. There was a statistically significant effect of light ($P < 0.0001$), which was increased when the lights were off. There were no significant interactions between factors, but there was a marginally significant interaction between selection and light ($P = 0.0597$). Light reduced movement to a greater extent in the control lines than in the selected lines. In general, there was an effect of selection that reduced movement in both sexes.

Discussion

This study reports data on two classes of data (body composition and movement) using *D. melanogaster* selected for starvation resistance. Different compounds and macromolecules that can be used for energy increased as a result of selection. Moreover, movement decreased as a result of selection for starvation resistance. Thus, two of the mechanisms proposed for adaptation to starvation, energy storage and energy conservation (Rion & Kawecki, 2007) were observed in the present study. In general, there was a negative correlation between abundance of energy-containing compounds and decreased movement.

Laboratory selection for starvation resistance results in energy compound changes in body composition

Body composition measurements from prior *D. melanogaster* artificial selection experiments for starvation have mainly focused on total lipid quantification; total lipid is often recorded as the difference between lean mass and dry weight within previous studies (Zwaan *et al.*, 1995; Chippindale *et al.*, 1996, 1998; Djawdan *et al.*, 1998; Harshman *et al.*, 1999a,b; Baldal *et al.*, 2006). These studies have established that selection for starvation resistance resulted in an increase in total lipid levels. In terms of a specific class of lipids, TAGs levels were observed to increase in response to selection for *D. melanogaster* starvation resistance (Harshman *et al.*, 1999a,b). Prior to the present study, it was not known how a range of energy-containing molecules and macromolecules respond to selection for starvation resistance.

Table 4. Male mean movement (SE) is the average number of times a laser beam in a tube is broken in a 10-min interval. Movement is the grand mean of the values (average value for all selected lines and the average value for all control lines, or average value of starved vs. unstarved flies, or average value for lights off vs. lights on).

Selection	Condition	Light	Mean (SE)
Control			5.66 (0.30)
Selected			4.09 (0.30)
	Starved		5.12 (0.32)
	Unstarved		4.64 (0.28)
		Off	5.74 (0.21)
		On	4.02 (0.21)

Table 5. Female mean movement (SE) is the average number of times a laser beam in a tube is broken in a 10-min interval. Movement is the grand mean of the values (average value for all selected lines and the average value for all control lines, or average value of starved vs. unstarved flies, or average value for lights off vs. lights on).

Selection	Condition	Light	Mean (SE)
Control			3.54 (0.20)
Selected			3.27 (0.20)
	Starved		3.29 (0.18)
	Unstarved		3.52 (0.18)
		Off	3.71 (0.15)
		On	3.10 (0.15)

In this study, various energy-containing molecules were measured including triglycerides, glycogen, total sugars and trehalose. The level of soluble protein was additionally measured because under extreme starvation conditions protein can be used for energy by some vertebrates. In addition, the ratio of TAGs, DAGs and FFAs to polar lipids (phospholipids) was estimated. Triglycerides and total sugars increased in both sexes as a result of selection. Male triglycerides (energy storage lipids) are appreciably lower than in females, but males gained a greater proportion of triglycerides as a result of selection (Tables 1 and 3). In males, trehalose and DAGs also increased as a response to selection. The response to laboratory selection is notable in that a range of energy-containing molecules increased in response to selection. This observation raises the question of whether a general regulator of sugar and lipid metabolism underlies the response to selection.

Body composition responses to the selective agent can predict the response to selection

Various macromolecules and compounds decreased markedly after starvation. This included triglycerides, diglycerides, glycogen, trehalose, total sugar and protein in males. Most of these compounds increased in response to selection for starvation resistance. Previously, the hypothesis of counter-impact selection was described (Harshman & Schmid, 1998); the argument was that the physiological consequences of the environmental perturbation (the selective agent) were compensated by selection, which acts to physiologically counter the impact of selection. For example, starvation reduces the levels of triglycerides and selection for starvation resistance results in an increase in triglycerides. Based on this study, a counter-impact response to selection could include other energy-containing molecules including sugars. The hypothesis of counter-impact selection was not entirely supported in this study as the effect of selection was not to oppose the effect of starvation for each class of energetic compound or macromolecule. Glycogen was especially noticeable in this regard because it markedly decreased in response to starvation, but did not increase as a correlated response to selection for starvation resistance. A similar starvation phenotypic perturbation vs. selection pattern for glycogen was observed in Harshman *et al.* (1999a,b).

Some macromolecules did not decrease in abundance as a result of starvation.

Soluble protein decreased in starved males, but not in starved females. Relevant data in this study show that triglyceride stores are appreciably lower in males than in females. Thus, males might reach a threshold of lipid depletion sooner than females, resulting in males metabolizing proteins faster under starvation conditions. This could be the reason that soluble protein decreases in males, but not females, in response to starvation. The response of FFAs to starvation and selection is interesting. Starvation did not result in a decrease in FFAs in this study nor did selection result in an increase in these lipid macromolecules. It is not apparent why FFAs are relatively invariant in the context of this study. However, in excess fatty acids become toxic, which could constrain maximum concentrations, whereas minimum levels could reflect the necessity of FFA availability to provide energy for life functions.

Decreased movement as an indirect response potentially relevant to energy conservation

The indirect effect of selection on movement is a novel consideration in the context of laboratory selection for starvation resistance. Movement has been measured in *D. melanogaster* selected for desiccation resistance, in which case there was no associated reduction in movement in the selected lines (Williams *et al.* 2004). In the present study, movement in males selected for starvation resistance was statistically lower and the trend of reduced movement was similar in females. This observation suggests that flies are conserving energy for starvation survival by moving less.

Metabolic rate is related to the degree of movement. It has been argued that decreased metabolic rate contributes to adaptation to environmental stress (Hoffman & Parsons, 1991). Studies conducted on a range of species have noted a reduction in metabolic rate in animals with high levels of stress resistance (for example, Lighton & Bartholomew, 1988). However, when the non-metabolizing mass (chiefly lipid) was removed as part of data normalization, evolved elevated stress resistance was not associated with increased metabolic rate (Djawdan *et al.*, 1997) in a *Drosophila* artificial selection experiment. Moreover, the per fly metabolic rate in starvation resistant lines was not reduced in selected lines compared to control lines (Harshman & Schmid, 1998) in *D. melanogaster* investigated in a laboratory selection experiment for starvation resistance. The implication of these studies is that energy conservation is not a mechanism for adaptation to starvation in laboratory-selected lines. However, this study indicated that energy conservation may underlie a portion of the response to selection based on the observation of decreased movement in the selected lines.

The effect of starvation on movement is additionally of interest. In other studies, starvation resulted in increased movement (Rion & Kawecki, 2007). In the present study, there was a statistically nonsignificant tendency for an increase in movement in starved males and statistically nonsignificant tendency for a decrease in movement in starved females. The effect of starvation on movement was not significant for males or females, and there was no consistency in how starvation affected movement in the two sexes.

The evolution of starvation resistance in the laboratory and natural populations

This study was a laboratory selection experiment and thus not directly relevant to natural selection in *Drosophila* or other populations. However, there could be relevance to selection for starvation resistance in natural populations of flies and humans. For example, *D. melanogaster* selected for desiccation resistance in the laboratory has been compared to drought-tolerant species in the field (Gibbs 2002). Some evolved responses in the laboratory were similar to adaptations in the field; for example, flies in both cases lose less water during respiration. In this study, energy storage lipids and sugars increased in abundance, and movement decreased, in response to selection. It will be interesting to determine whether these traits exhibit a similar pattern of quantitative genetic variation in natural *Drosophila* populations and genetically covary when there is natural selection for starvation resistance in the field.

There could be relevance to human populations which is described here as speculation. This can be an important part of thinking broadly about the implications of the experimental results. In humans, the joint occurrence of obesity, type 2 diabetes, hyperlipidemia and hypertension has increased in prevalence throughout the world, resulting in increased mortality. This has lead to the widespread view that these diseases contribute to one syndrome, classified medically as the metabolic syndrome (Wilson & Grundy, 2003). The elevated lipid and sugar is caused by the intake of food. Increased body weight is correlated with decreased movement in humans, which presumably is not an energy conservation mechanism. However, the propensity to respond to increased food consumption, both in terms of the metabolic syndrome and movement, could have a common genetic basis perhaps driven by regular selection for resistance to starvation in human populations throughout their evolution. There is evidence for a significant genetic contribution to the metabolic syndrome (Pollex & Hegele, 2006). However, the genetic architecture of the metabolic syndrome as a complex trait, and related traits, is poorly understood.

This study could be relevant to understanding metabolic syndrome and related traits in humans. In this study, triglycerides and sugars increased in abundance as a result of selection whereas movement decreased as a result of selection. The response to selection could be to some degree modular such that a genetic-based module mediates increased energy storage lipid, increased sugars and decreased movement. The response to selection in this study should be investigated in terms of the underlying genetic architecture in this model for the study of genetics and genomics may provide for an understanding of evolved starvation resistance as proxy for obesity and related phenotypes.

Conclusions

Within this study, selection for starvation resistance increased the abundance of triglycerides and sugars. Energy accumulation is one mechanism for resisting starvation and the response affects a range of relevant compounds and macromolecules. Decreased movement was observed, which suggests an energy conservation response to selection. Overall, two mechanisms for survival under starvation conditions have evolved in the laboratory with potential relevance to natural populations.

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