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RESEARCH ARTICLE

Starvation resistance is associated with developmentally specified changes in sleep, feeding and metabolic rate

Elizabeth B. Brown[1], Melissa E. Slocumb[1], Milan Szuperak[2], Arianna Kerbs[1], Allen G. Gibbs[3], Matthew S. Kayser[2] and Alex C. Keene[1,*]

ABSTRACT

Food shortage represents a primary challenge to survival, and animals have adapted diverse developmental, physiological and behavioral strategies to survive when food becomes unavailable. Starvation resistance is strongly influenced by ecological and evolutionary history, yet the genetic basis for the evolution of starvation resistance remains poorly understood. The fruit fly *Drosophila melanogaster* provides a powerful model for leveraging experimental evolution to investigate traits associated with starvation resistance. While control populations only live a few days without food, selection for starvation resistance results in populations that can survive weeks. We have previously shown that selection for starvation resistance results in increased sleep and reduced feeding in adult flies. Here, we investigate the ontogeny of starvation resistance-associated behavioral and metabolic phenotypes in these experimentally selected flies. We found that selection for starvation resistance resulted in delayed development and a reduction in metabolic rate in larvae that persisted into adulthood, suggesting that these traits may allow for the accumulation of energy stores and an increase in body size within these selected populations. In addition, we found that larval sleep was largely unaffected by starvation selection and that feeding increased during the late larval stages, suggesting that experimental evolution for starvation resistance produces developmentally specified changes in behavioral regulation. Together, these findings reveal a critical role for development in the evolution of starvation resistance and indicate that selection can selectively influence behavior during defined developmental time points.

KEY WORDS: Selection, Development rate, Food consumption, *Drosophila*

INTRODUCTION

Food acquisition represents a major challenge to many animal species, and the ability to locate food, or survive in the absence of food, strongly associates with reproductive fitness (Chippindale et al., 1996; Wayne et al., 2006). The ability to resist starvation varies dramatically throughout the animal kingdom, and even between closely related species, yet surprisingly little is known about the biological basis for differences in this behavior (Gibbs and Reynolds, 2012; Matzkin et al., 2009; Rion and Kawecki, 2007). Animals have evolved diverse mechanisms for responding to acute shortages in nutrient availability, including the induction of foraging behavior, alterations in sleep and locomotor activity, and changes in metabolic rate (Schmidt, 2014; Stahl et al., 2017; Sternson et al., 2013; Yurgel et al., 2014). While starvation resistance is likely influenced by developmental processes that contribute to an organism's size, metabolic phenotypes and brain function, it is not known whether selection occurs at developmentally specified stages or is maintained throughout development. Defining the effects of selection for starvation resistance on behavior and metabolism across development is therefore critical for understanding the developmental specificity of evolved changes in these processes.

The fruit fly *Drosophila melanogaster* provides a powerful model for investigating the mechanistic basis of starvation resistance (Rion and Kawecki, 2007). Outbred populations of fruit flies display highly variable starvation resistance, as well as traits that are associated with starvation resistance, including developmental timing, sleep and feeding behaviors (Folguera et al., 2008; Garlapow et al., 2016; Harbison et al., 2017; Masek et al., 2014; Svetec et al., 2015; Yadav and Sharma, 2014), but little is known about how these individual traits contribute to the evolution of starvation resistance. We have implemented experimental evolution by starving outbred adult *Drosophila* until only 15% of the initial population remain alive, then passaging the survivors onto the next generation (Hardy et al., 2018). These populations have been independently selected over 100 generations, resulting in flies that survive up to two weeks in the absence of food, while non-selected flies survive for only 3–4 days. These starvation-selected populations provide an opportunity to examine how behavioral and physiological traits are altered by selection for starvation resistance and whether selection in adults also influences their development.

Altered life history and behavioral changes in adults are associated with evolutionarily acquired resistance to nutrient stress (Bubliy and Loeschcke, 2005; Gefen et al., 2006; Kolss et al., 2009), but the specific contributions of the many behavioral and physiological changes to starvation resistance have been difficult to test experimentally. We have previously identified increased sleep and reduced feeding in adult *Drosophila* selected for resistance to starvation stress (Masek et al., 2014; Slocumb et al., 2015). While these traits likely emerged as a mechanism to conserve energy in the absence of food, their precise roles in starvation resistance are unknown. In addition, both sleep and feeding are developmentally plastic behaviors, and are modulated by both shared and independent neural mechanisms during the larval and adult stages (Itskov and Ribeiro, 2013; Koh et al., 2006; Melcher and Pankratz, 2005; Pool and Scott, 2014; Szuperak et al., 2018). *Drosophila* eat voraciously throughout development, and this is essential for organismal growth and the generation for energy stores that persist through adulthood (Tennessen and Thummel, 2011). In addition,

[1]Department of Biological Sciences, Florida Atlantic University, Jupiter, FL 33458, USA. [2]Departments of Psychiatry and Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA. [3]Department of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154, USA.

*Author for correspondence (KeeneA@FAU.edu)

A.C.K., 0000-0001-6118-5537

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we have recently characterized larval sleep and found that this sleep is critical for development (Szuperak et al., 2018). Therefore, it is possible that selection for starvation resistance differentially influences adult behavior and physiological function, or that shared genetic architecture between development and adulthood results in an evolutionary constraint on developmental state-specific modification of behavior.

Here, we investigate sleep, feeding and metabolic function during development in flies selected for starvation resistance. We find that development time is extended, starting at the second instar larval stage, and persists throughout development. In addition, whole-body metabolic rate is reduced during both development and adulthood and is accompanied by an increase in mass, suggesting that reduced energy expenditure allows these starvation-resistant populations to increase their energy stores. Our findings also reveal that increased sleep and reduced feeding are specific to the adult stage, suggesting that selection for starvation resistance can target specific behaviors at different developmental time points.

MATERIALS AND METHODS

Drosophila maintenance and fly stocks

Starvation-selected populations at generation 122 were obtained and then tested and maintained off-selection for a maximum of 5 generations. All populations were grown and maintained on standard *Drosophila* medium (Bloomington *Drosophila* Stock Center) and maintained in incubators (Percival Scientific, Perry, IA, USA) at 25°C and 50% humidity on a 12 h light:12 h dark cycle. For larval experiments, adult flies were maintained in population cages with access to grape juice agar and yeast paste (Featherstone et al., 2009). Unless stated otherwise, eggs were collected from the cages within 2 h of being laid and then transferred into Petri dishes containing standard food at a constant density of 100 eggs per dish.

Development time

Eggs were transferred into Petri dishes containing standard food and green food coloring, which allowed for easier viewing of the larvae, at a density of 25 eggs per dish. Larvae were then scored every 4 h for their transition through the first, second and third instar stages. Each larval stage was distinguished by the size and complexity of their mouthparts (transition from first to second instar), as well as the branching pattern of the anterior spiracles and the size of the dark orange ring on the posterior spiracles (transition from second to third instar; JoVE Science Education Database). The time at which at least 50% of the larvae within each Petri dish had transitioned through each developmental stage was recorded. Time to pupariation and eclosion was measured independently from each larval instar stage. Eggs were collected within 2 h of being laid and placed individually into glass test tubes, each containing 2 ml standard *Drosophila* medium. Tubes were then scored every 4 h.

Feeding behavior

Short-term food intake in adult flies was measured as previously described (Wong et al., 2009). Briefly, sets of five 3- to 4-day-old female flies were either transferred to vials containing a damp Kimwipe and starved, or maintained on standard food for 24 h. At zietgeber time (ZT) 0, flies from both treatments were transferred to food vials containing 1% agar, 5% sucrose and 2.5% blue dye (Federal Food, Drug and Cosmetic Act, blue dye no. 1, Spectrum Chemical Manufacturing Corp., New Brunswick, NJ, USA). After 30 min, flies were flash frozen and stored for subsequent analyses. For food consumption measurements in larvae, eggs were obtained as previously described. Eggs were transferred to Petri dishes containing standard food at a larval density of 100 larvae per dish. Food consumption was measured at 60 and 96 h after egg laying for second and third instar larvae, respectively, as previously described (Kaun et al., 2007). Briefly, larvae were transferred to Petri dishes containing a thin layer of 1% agar and yeast paste with 2.5% blue dye. After 15 min of feeding, larvae were collected and then washed in ddH_2O three times. The larvae were then flash frozen in groups of 10 and 5 for second and third instar larvae, respectively. Each larval and adult sample was homogenized in 400 µl phosphate-buffered saline (PBS) and then centrifuged at 4°C at 15,710 ***g***. The supernatant was then extracted and its absorbance at 655 nm was measured in triplicate using a 96-well plate absorbance spectrophotometer (Bio-Rad Laboratories). Baseline absorbance was determined by subtracting the absorbance obtained from flies/larvae not fed blue dye from each experimental sample. The amount of food consumed was then determined from a standard curve. To assess feeding rate in second and third instar larvae, the number of mouth hook contractions were counted (Shen, 2012). For each group, second or third instar larvae were placed onto a Petri dish containing agar and yeast paste. After a 1 min acclimation period, larvae were video recorded and the number of mouth hook contractions within a 30 s period were counted.

Mass

For adults, 3- to 5-day-old female flies were isolated and placed on fresh medium for 24 h, and then the mass of groups of 10 flies were determined. For second and third instar larvae, mass was measured at 60 and 96 h after egg laying and was determined using groups of 10 and 20 larvae, respectively.

Sleep and waking activity

In adults, individual 3- to 5-day-old mated female flies were placed into tubes containing standard food and allowed to acclimate to experimental conditions for at least 24 h. Sleep and activity were then measured over a 24 h period starting at ZT0 using the *Drosophila* Locomotor Activity Monitor System (DAMs) (Trikinetics, Waltham, MA, USA) as previously described (Hendricks et al., 2000; Shaw et al., 2000). The DAM system measures activity by counting the number of infrared beam crossings for each individual fly. These activity data were then used to calculate bouts of immobility of 5 min or more using the *Drosophila* sleep counting macro (Pfeiffenberger et al., 2010), from which sleep traits were then extracted. In larvae, sleep and activity was measured as described (Szuperak et al., 2018). Briefly, individual freshly molted second instar larvae were loaded into wells of custom-made polydimethylsiloxane (PDMS) microplates (‘larva lodges’) containing 3% agar and 2% sucrose with a thin layer of yeast paste. Larva lodges were loaded into incubators at 25°C and time-lapse images were captured every 6 s under dark-field illumination using infrared LEDs. Images were analyzed using custom-written MATLAB software and activity/quiescence determined by pixel value changed between temporally adjacent images. Total sleep was summed over 6 h beginning 2 h after the molt to second instar. Sleep bout number and average sleep bout duration was calculated during this same period.

Starvation resistance

After sleep assessment, the same flies were also used to measure starvation resistance. Following 24 h of testing on standard food, flies were transferred to tubes containing 1% agar (Fisher Scientific) and starvation resistance was assessed. The time of death was manually determined for each individual fly as the last bout of waking activity.

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Metabolic rate

Metabolic rate was measured using indirect calorimetry by measuring CO_2 production (Stahl et al., 2017). Staged larvae were placed in groups of five (second instar) or individually (third instar) onto a small dish containing standard food medium. Each dish was placed in a behavioral chamber where larvae were acclimated for 30 min, which is approximately the time required to purge the system of ambient air and residual CO_2. Metabolic rate was then assessed by quantifying the amount of CO_2 produced in 5 min intervals for 1 h. All experiments were conducted during ZT0–ZT6 to minimize variation attributed to circadian differences in sleep, feeding or metabolic rate. Metabolic rate in adults was assessed as described previously (Stahl et al., 2017). Briefly, adult flies were placed individually into behavioral chambers containing a food vial of 1% agar and 5% sucrose. Flies were acclimated to the chambers for 24 h and then metabolic rate was assessed by quantifying the amount of CO_2 produced in 5 min intervals during the subsequent 24 h. Metabolic data for each group were normalized for body weight by dividing metabolic rate by mass, measured as described above.

Statistical analysis

To assess differences in survivorship between starvation-selected and control populations, starvation resistance was analyzed using a log-rank test. Log-rank tests were also used to assess differences in development time, from first instar to eclosion. A two-way ANOVA was performed on measurements of metabolic rate, mass, food consumption, mouth hook contractions and sleep traits (factor 1: selection regime; factor 2: replicate population). If significant differences were observed, Sidak's multiple comparisons test was performed to identify significant differences within each replicate population. All statistical analyses were performed using GraphPad Prism 7.0. In all figures, individual data points are shown, along with bars indicating mean values and error bars showing s.e.m.

RESULTS

Selection for starvation resistance in *Drosophila*

To assess developmental correlates of increased starvation stress, we utilized outbred populations that were subjected to laboratory natural selection for starvation resistance (Rose et al., 1996). Flies were selected for starvation resistance by placing adult flies on agar and passaging starvation-resistant populations onto food when only ∼15% of flies remained alive. Three parallel starvation-resistant groups were generated (S_A, S_B and S_C) as well as three controls that were continuously passaged on food (F_A, F_B and F_C). Experiments in this study utilized flies maintained on this selection protocol for 122 generations (Fig. 1A). In agreement with previous studies performed on flies selected for 60–80 generations (Hardy et al., 2018; Masek et al., 2014), this selection protocol robustly increased starvation resistance. All three S populations survived on agar for an average of 9–13 days compared with 2–3 days for the F populations (Fig. 1B,C), confirming that selection for starvation resistance results in a ∼4-fold increase in survival under starvation conditions.

It has been previously shown that starvation selection is associated with a larger body size in adult flies (Masek et al., 2014; Slocumb et al., 2015). To investigate whether this increase in body mass also occurs during development or is restricted to adults, we measured body mass during the second and third instar stages. Overall, we found that starvation-selected populations weighed significantly more than control populations at the second instar stage. However, we did not observe this effect when directly comparing each replicate F and S group individually (Fig. 1D). In the third instar stage, starvation-selected populations weighed significantly more than fed control populations, and each individual S group replicate weighed significantly more than their respective F control group (Fig. 1E). This increase in mass for all three starvation-selected replicate groups was maintained into adulthood (Fig. 1F). These findings suggest that starvation selection is accompanied by an increase in mass that occurs during the third instar stage and persists through adulthood.

Starvation selection increases development time

It is possible that delayed development contributes to starvation resistance by allowing flies to accumulate energy stores during the larval stages. To determine whether the rate of development is altered by starvation selection, we measured the time from egg laying to each developmental transition. Overall, development rate was delayed across all S groups, confirming that starvation selection increases development time (Fig. 2A). A direct comparison of each developmental stage revealed no difference between F control groups and starvation-selected S groups in the transition from egg to first instar larvae, suggesting the selection protocol does not affect the earliest stages of development (Fig. 2B). Development time was significantly delayed at all subsequent developmental stages (from first instar to pupariation) when the three replicate starvation-selected populations and three control populations were pooled (Fig. S1). However, *post hoc* analyses on development time at each of these developmental stages revealed population-specific effects on the time spent within each stage. As such, a direct comparison of each replicate group revealed no differences in the duration of time spent as first instar larvae (Fig. 2C). For the duration of time spent as second instar larvae, a similar comparison of each replicate group revealed that significant differences were only observed between the F_B and S_B populations (Fig. 2D). In contrast, all three S group replicate populations spent significantly longer in the third instar stage (Fig. 2E). During pupariation, significant differences in development time were again only observed between the F_B and S_B populations (Fig. 2F). Therefore, delayed development time is present across all starvation-selected populations, but is particularly robust in the S_B population. Overall, these findings raise the possibility that increased body size and starvation resistance are related to delayed development.

Starvation selection decreases metabolic rate

In addition to delayed development, reduced metabolic rate provides a mechanism for conserving energy (Dulloo and Jacquet, 1998; Ma and Foster, 1986). Animals, including *Drosophila*, reduce metabolic rate under starvation conditions (Blaxter, 1989; McCue, 2010; Wang et al., 2006), suggesting that modulation of metabolic rate may promote starvation resistance. To determine the effect of starvation selection on metabolic rate, we used indirect calorimetry to determine CO_2 release, a proxy for metabolic rate, in both larvae and adults. Measurements of metabolic rate were then normalized to body mass in order to account for differences in body size between the F control groups and starvation-selected S groups. The system used to measure metabolic rate is highly sensitive, and has previously been used to detect CO_2 release from single flies (Fig. 3A; Stahl et al., 2017). In second instar larvae, no changes in metabolic rate were detected between F control groups and starvation-selected S groups (Fig. 3C). However, metabolic rate was reduced in third instar larvae when the three replicate starvation-selected populations and three control populations were each pooled (Fig. S1). *Post hoc* analyses of each replicate population revealed a significant decrease in metabolic rate in the S_A and S_C populations compared with their respective F control populations (Fig. 3D). In

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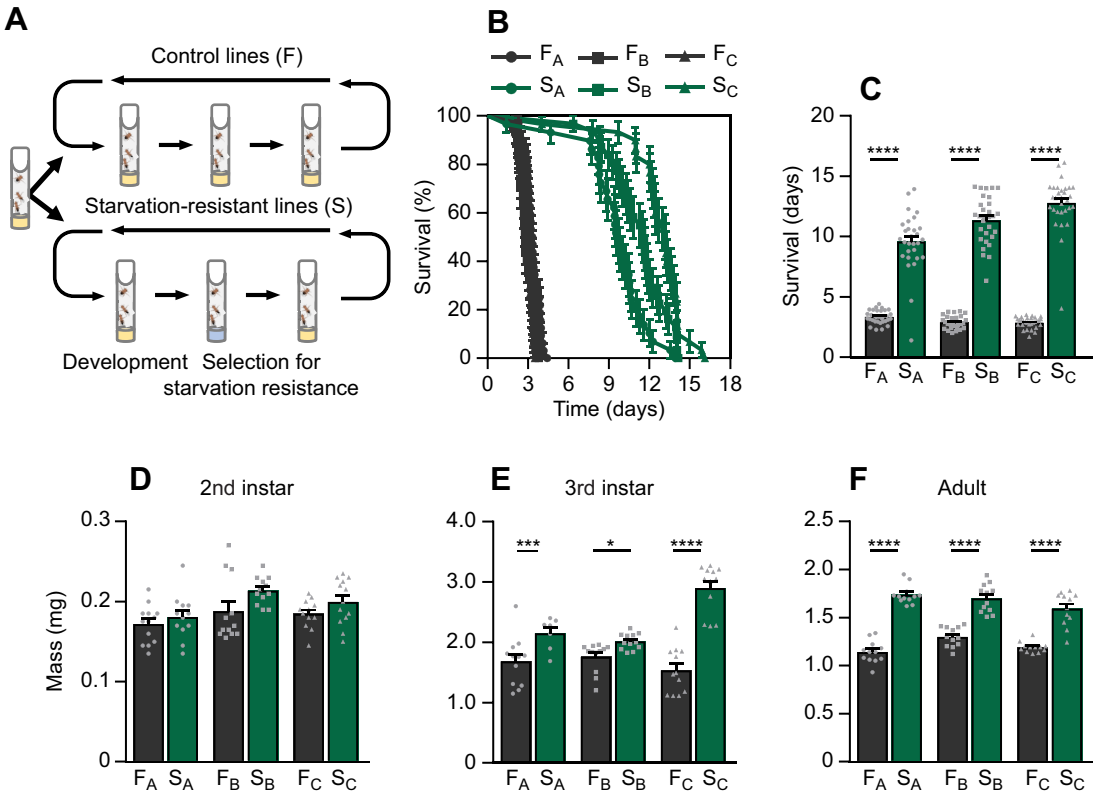

Fig. 1. Selection for starvation resistance is correlated with larger body size. (A) Flies were selected for starvation resistance by maintaining adult flies on agar until ∼15% of flies remained alive. Three starvation-resistant groups were generated in addition to three fed control groups. (B,C) The S populations (S_A, S_B and S_C) survive significantly longer on agar than the F populations (F_A, F_B and F_C) (log-rank test: χ^2=210.7, d.f.=1, P<0.001; S and F populations pooled). (B) Survivorship curves showing the percentage of flies remaining alive as a function of the duration of starvation. (C) Mean survivorship of the S and F populations. Survivorship was measured once flies were transferred to agar. N=27–32 per population. (D) Selection for starvation resistance increases mass in second instar larvae (two-way ANOVA: $F_{1,66}$=6.52, P=0.0130, N=12 per population). However, *post hoc* analyses revealed no significant differences among replicate populations (A: P=0.8937; B: P=0.0681; C: P=0.3828). (E) Selection for starvation resistance increases mass in third instar larvae (two-way ANOVA: $F_{1,66}$=83.7, P<0.0001, N=12 per population) and occurs in all three replicate populations (A: P=0.0003; B: P=0.0186; C: P<0.0001). In addition, we found that measurements of mass in third instar larvae were population specific ($F_{2,66}$=8.645, P=0.0005) and that there was a significant interaction between mass and population ($F_{2,66}$=16.88, P<0.0001). (F) Selection for starvation resistance increases mass in adults (two-way ANOVA: $F_{1,66}$=266, P<0.0001, N=12 per population), and occurs in all three replicate populations (A: P<0.0001; B: P<0.0001; C: P<0.0001). Similarly to third instar larvae, we found that measurements of mass in adults were population specific ($F_{2,66}$=4.954, P=0.0099) and there was a significant interaction between mass and population ($F_{2,66}$=5.125, P<0.0001). Error bars represent s.e.m. Here and in subsequent figures, asterisks indicate the level of significance: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

adult flies, metabolic rate was significantly decreased in all three starvation-selected populations (Fig. 3E). Therefore, selection for starvation resistance results in reduced metabolic rate that commences during the third instar stage and persists into adulthood.

Differential effects of starvation selection on feeding and sleep

We have previously shown that food consumption is reduced in fasted starvation-selected adult flies (Masek et al., 2014). However, the effects of selection on larval feeding remain unknown. To quantify feeding in second and third instar larvae, we measured food intake by placing flies on yeast paste laced with blue dye. The amount of food consumed over a 15 min period was then measured based on spectrophotometric analysis of dye consumed during this time period. Food consumption was significantly increased among second and third instar larvae when the three replicate starvation-selected populations and three control populations were each pooled (Fig. S1). However, during the second instar stage, *post hoc* analyses revealed that this effect was only significant in the F_C and S_C groups (Fig. 4A,B). During the third instar stage, food consumption was increased in all three starvation-selected replicate populations (Fig. 4C,D), suggesting that starvation selection promotes larval feeding. In contrast to larval feeding behavior, no differences were observed in food consumption across all three populations of starvation-selected adult flies in the fed state (Fig. 4E,F). However, when animals were food deprived, food consumption was significantly reduced across all three starvation-selected populations to induce a robust feeding response (Fig. 4G,H). These findings suggest that selection for starvation resistance has different effects on food consumption during the larval and adult stages.

It is possible that increased food consumption in starvation-selected larvae is a result of increased feeding drive or is secondary to their overall larger body size. To differentiate between these possibilities, we measured feeding rate by calculating the number of mouth hook contractions over a 30 s period. The number of mouth hook contractions did not differ between starvation-selected and control populations for second or third instar larvae (Fig. S2).

We previously reported that selection for starvation resistance increases sleep in adults (Masek et al., 2014). Here, we confirmed these results, finding that sleep duration was increased in all three starvation-selected populations, which is a consequence of increased bout length but not bout number (Fig. 5A–D). These results raise the possibility that energy conservation as a result of

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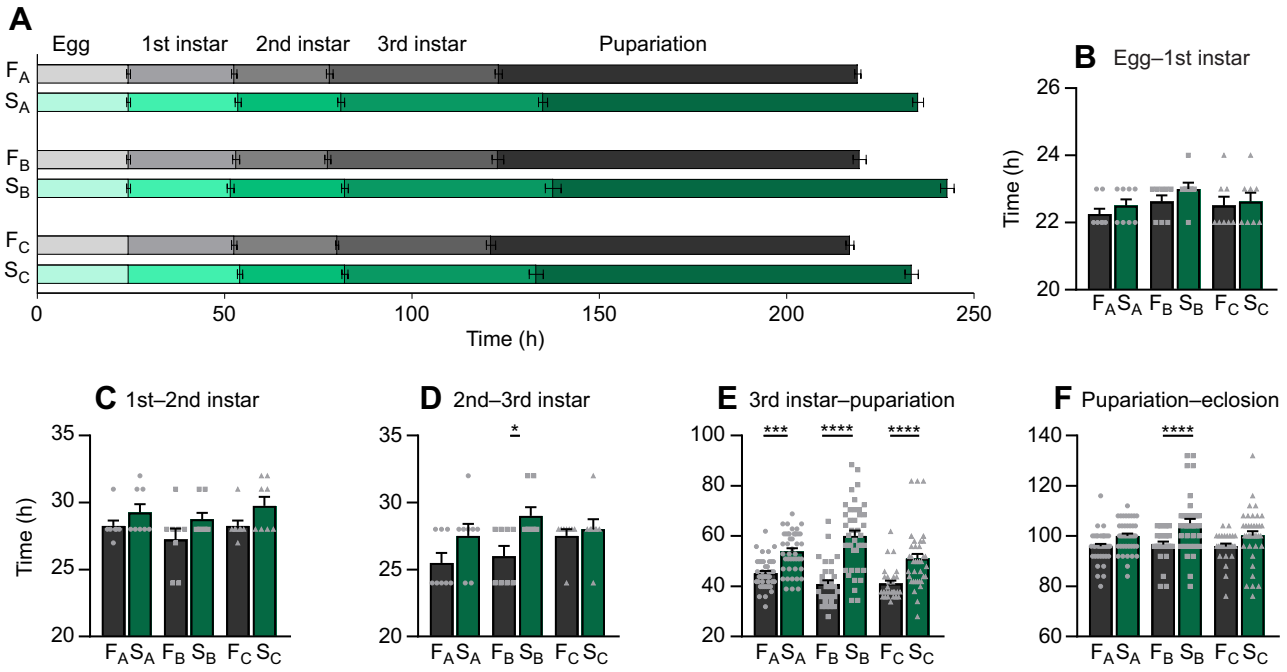

Fig. 2. Selection for starvation resistance extends each stage of the *Drosophila* life cycle. (A) Average time spent in each stage of the life cycle from egg to eclosion. Increasingly darker bars indicate progression to later stages of larval and pupal development. (B) Average time it takes to hatch as a first instar larva. The S populations take equally as long to hatch as the F populations (two-way ANOVA: $F_{1,42}$=2.066, P=0.1581). (C) Average time it takes to molt from first instar into second instar larvae. The S populations take longer to transition from first instar to second instar larvae relative to the F populations (two-way ANOVA: $F_{1,42}$=7.658, P=0.0084). However, *post hoc* analyses revealed no significant differences among replicate populations (A: P=0.5567; B: P=0.2199; C: P=0.2199). (D) Average time it takes to molt from second instar into third instar larvae. The S populations take longer to transition from second instar to third instar larvae relative to the F populations (two-way ANOVA: $F_{1,42}$=9.517, P=0.0036). However, *post hoc* analyses revealed significant differences within the B population only (A: P=0.1661; B: P=0.0170; C: P=0.9492). (E) Average time it takes for third instar larvae to begin pupariation. The S populations take longer to transition from third instar into prepupae relative to the F populations (two-way ANOVA: $F_{1,217}$=94.81, P<0.0001) in all three replicate populations (A: P<0.0001; B: P<0.0001; C: P<0.0001). (F) Average time from pupariation to eclosion. The S populations take longer to eclose from the pupal phase as adult flies relative to the F populations (two-way ANOVA: $F_{1,217}$=27.07, P<0.0001) in all three replicate populations (A: P=0.0521; B: P<0.0001; C: P=0.0893). Egg to third instar measurements: N=8; pupation and eclosion measurements: N=28–40. Error bars represent s.e.m.

increased sleep may also occur during the larval stages. Recently, sleep has been characterized in second instar *Drosophila* larvae, allowing for the characterization of changes in sleep throughout development (Fig. 5E; Szuperak et al., 2018). Overall, we found that sleep increases among starvation-selected second instar larvae when the three replicate starvation-selected populations and three control

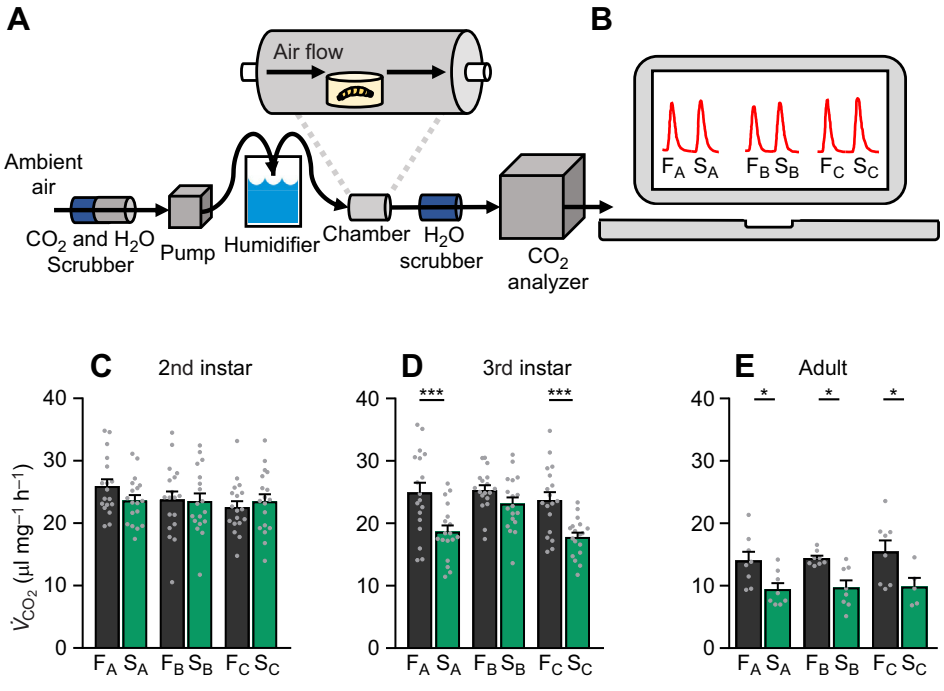

Fig. 3. Selection for starvation resistance decreases metabolic rate and occurs in the later stages of larval development. (A) Metabolic rate was measured in second and third instar larvae, and in adults. Measurements were taken using a stop-flow respirometry system that measured the amount of CO_2 produced over time. (B) Representative traces of each F and S population indicating the unadjusted amount of CO_2 produced within each experimental chamber over time. (C) There is no change in metabolic rate in second instar larvae (two-way ANOVA: $F_{1,102}$=0.3521, P=0.5543, N=18 per population). (D) In third instar larvae, selection for starvation resistance significantly decreases metabolic rate (two-way ANOVA: $F_{1,102}$=0.27.89, P<0.0001, N=18 per population). However, this effect is population specific (A: P=0.0004; B: P=0.4276; C: P=0.0008). (E) Metabolic rate is also significantly reduced in adults (two-way ANOVA: $F_{1,39}$=21.71, P<0.0001, N=4–6 per population) and persists in all replicate populations (A: P=0.0396; B: P=0.0318; C: P=0.0235). Error bars represent s.e.m.

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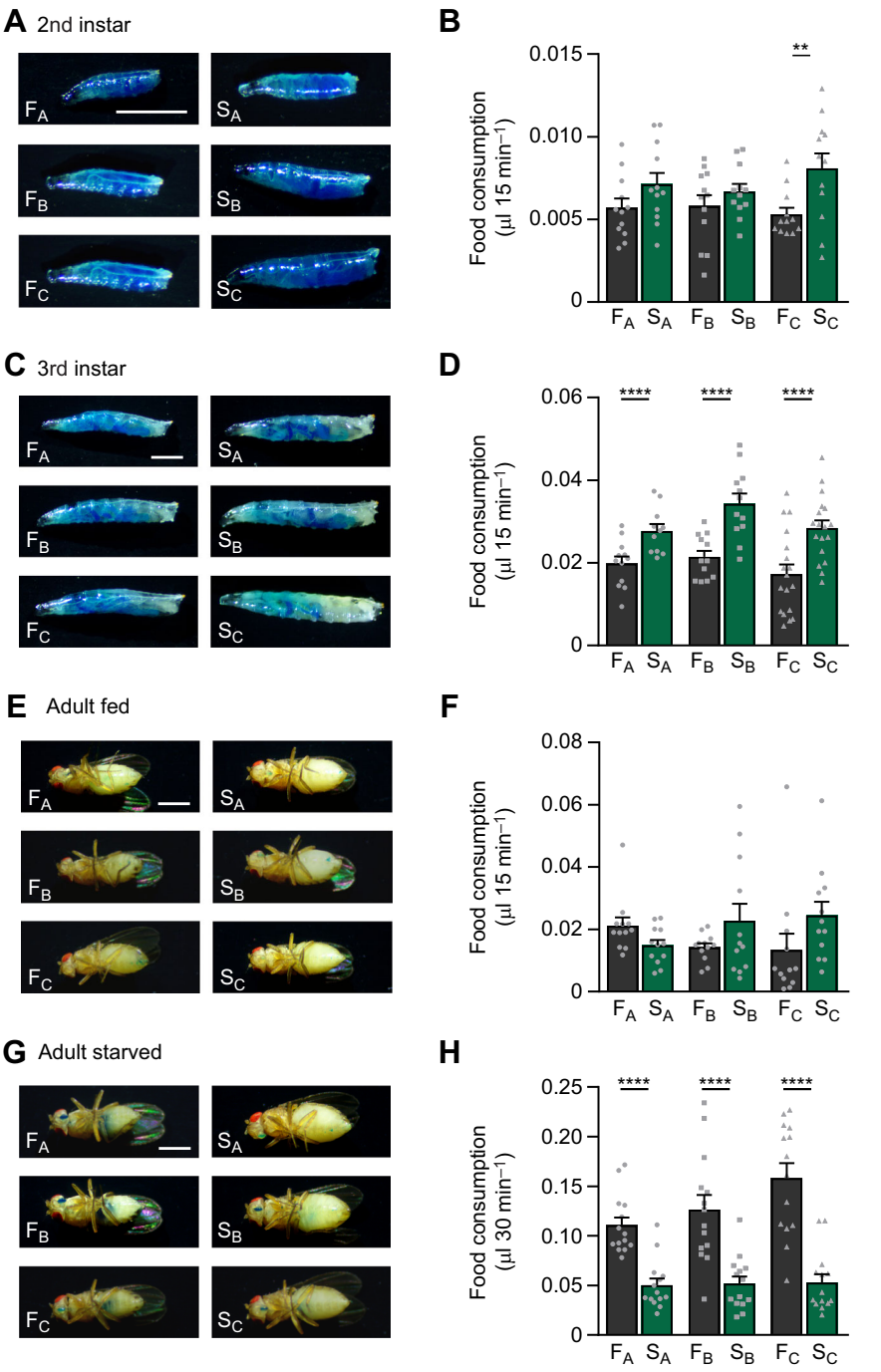

Fig. 4. Selection for starvation resistance is correlated with an increase in food consumption beginning in the third instar stage. (A) Representative second instar larva from each population after 15 min of feeding on yeast paste supplemented with 2.5% blue dye. (B) Overall, starvation-resistant populations consumed significantly more as second instar larvae (two-way ANOVA: $F_{1,66}$=10.68, P=0.0017, N=12 per population). However, *post hoc* analyses revealed that only the S_C group increased food consumption relative to its control (A: P=0.3158; B: P=0.6912; C: P=0.0088). (C) Representative third instar larva from each population after 15 min of feeding on yeast paste supplemented with 2.5% blue dye. (D) Starvation-resistant populations consumed significantly more as third instar larvae (two-way ANOVA: $F_{1,71}$=39.02, P<0.0001, N=12–18 per population) and *post hoc* analyses revealed that this is the case for all three replicate groups (A: P=0.0062; B: P=0.0002; C: P<0.0001). (E) Representative fed adult female from each population after 30 min of feeding on food medium supplemented with 2.5% blue dye. (F) Fed adults from starvation-resistant populations consumed the same as control populations (two-way ANOVA: $F_{1,66}$=1.996, P=0.1625, N=12 per population). (G) Representative starved adult female from each population after 30 min of feeding on food medium supplemented with 2.5% blue dye. (H) Adults from starvation-resistant populations consumed significantly less after 24 h of starvation than their respective controls (two-way ANOVA: $F_{1,78}$=86.21, P<0.0001, N=14 per population) and *post hoc* analyses revealed that this is the case for all three replicate groups (A: P=0.0004; B: P<0.0001; C: P<0.0001). Error bars represent s.e.m. Scale bars for all images: 0.5 mm.

populations were each pooled (Fig. S1). However, *post hoc* analyses revealed that when sleep was assessed in each replicate population, an increase in sleep was only observed among the F_C and S_C populations, suggesting that these differences in sleep are present throughout development (Fig. 5F). No significant differences in bout length or bout number were detected between replicate populations of second instar larvae, although a trend towards increased bout length in the S_C population was detected, suggesting that sleep architecture is largely unaffected by selection for starvation resistance (Fig. 5G,H). Although we found no differences in sleep in second instar larvae, it is possible that additional sleep differences exist in third instar larvae; however, it remains unknown whether third instar larvae exhibit sleep states.

DISCUSSION

Here, we report on the ontogenetically specified changes in behavior and metabolic rate induced by selection for starvation resistance. We found that starvation selection extends larval development beginning in the second instar stage, with a concomitant decrease in metabolic rate and increase in food consumption beginning in the third instar stage. In adults, however, metabolic rate remains low, while food consumption remains unchanged and sleep is increased. These results suggest that starvation selection has differential effects on behavioral and metabolic traits as development progresses from the larval stages into adulthood and is consistent with a strategy where starvation-selected larvae prioritize growth, while adults prioritize energy conservation.

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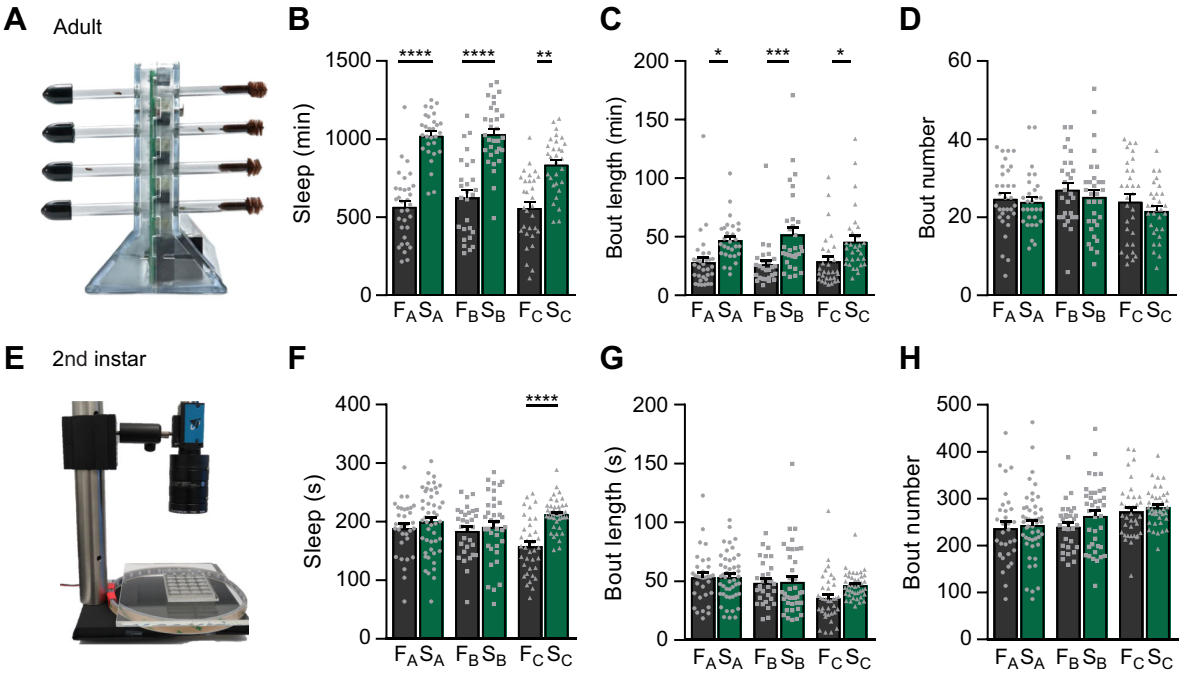

Fig. 5. Selection for starvation resistance increases sleep duration and occurs in adults only. (A) Sleep traits in adults were measured using the *Drosophila* activity monitoring system. (B) Starvation-resistant populations sleep significantly more as adults (two-way ANOVA: $F_{1,173}$=123.7, P<0.0001) and results are consistent across all three groups (A: P<0.0001; B: P<0.0001; C: P=0.0021). The magnitude of the increase in sleep duration was population specific, as there was a significant interaction between selection regime and line ($F_{2,173}$=4.889, P=0.0087). (C) This increase in sleep duration is a result of an increase in the length of each sleep episode (two-way ANOVA: $F_{1,173}$=29.87, P<0.0001) and is also consistent in all three groups (A: P=0.0110; B: P=0.0004; C: P=0.0288). (D) However, the number of sleep episodes does not differ (two-way ANOVA: $F_{1,173}$=1.659, P=0.1994). (E) Sleep traits in larvae were measured using custom-made larva lodges. (F) Starvation-resistant populations sleep significantly more as larvae (two-way ANOVA: $F_{1,220}$=14.5, P=0.0002). However, *post hoc* analyses revealed that only the S_c population increased sleep relative to its control (A: P=0.6872; B: P=0.9047; C: P<0.0001). The length of each sleep episode does not differ (G; two-way ANOVA: $F_{1,220}$=2.351, P=0.1266) nor does the number of sleep episodes (H; two-way ANOVA: $F_{1,1220}$=2.304, P=0.1305). Adults: N=26–32; larvae: N=31–48. Error bars represent s.e.m.

Previous studies have found that selection for starvation resistance results in slower development in *Drosophila* (Chippindale et al., 1996; Hoffmann and Harshman, 1999; Masek et al., 2014; Reynolds, 2013), suggesting that extended larval development represents a mechanism for developing starvation resistance as adults. Here, we showed that this reduced development rate begins as early as the second instar stage and persists until eclosion. During development, standard laboratory strains of *D. melanogaster* larvae increase their body size ∼200-fold during the ∼4 days of larval development (Church and Robertson, 1966), raising the possibility that even subtle changes in development rate may significantly affect adult body size and energy stores. Progression through each larval transition is regulated by the steroid hormone 20-hydroxyecdysone, a master regulator of developmental timing (Riddiford, 1993; Liu et al., 2017; Yamanaka et al., 2013) and it is possible that starvation selection acts to modify expression of this hormone, thereby delaying the onset of each larval transition. Additionally, several genetic factors have been identified that regulate nutrient-dependent changes in developmental timing, including the target of rapamycin signaling pathway (Colombani et al., 2003; Layalle et al., 2008) and insulin-like peptides (Ikeya et al., 2002; Slaidina et al., 2009). Although significant advances have been made in elucidating the mechanisms underlying larval development and growth, our understanding of how environmental conditions, including starvation, can modulate these factors remain poorly understood. Our study reveals that environmental stressors can be potent selective forces that have a strong impact on the timing of larval growth and development.

It is proposed that animals develop resistance to starvation stress by reducing energy expenditure (Aggarwal, 2014; Hoffmann and Parsons, 1989; Marron et al., 2003; Rion and Kawecki, 2007). Here, we show that metabolic rate is reduced in third instar larvae, as well as in adults, across all starvation-selected populations tested. While to our knowledge, the metabolic rate of *Drosophila* starvation-resistant larvae has not previously been studied, earlier reports examining metabolic rate in adults from different populations of *D. melanogaster* selected for starvation stress found no effect of selection on metabolic rate in flies (Baldal et al., 2006; Djawdan et al., 1997; Harshman and Schmid, 1998). However, there is evidence that selection for starvation resistance results in the production of different metabolic enzymes in response to starvation (Harshman and Schmid, 1998) as well as an accumulation of energy stores (Masek et al., 2014; Schwasinger-Schmidt et al., 2012; Slocumb et al., 2015). In our study, we measured the metabolic rate of adult flies over a 24 h period, thereby including any potential variation in the circadian effects of feeding, sleep and metabolic rate. It is possible that the independent origins of the selected populations resulted in selection on metabolic rate-dependent and -independent pathways, leading to enhanced starvation resistance. However, our finding that metabolic rate is reduced in multiple independent lines of starvation-selected populations suggests that these differences may be attributed to the initial outbred populations of flies used to evolve starvation resistance.

Increased body size is a fitness-related trait that promotes tolerance to stress (Ewing, 1961). As such, environmental perturbation and food shortages may uniquely affect fitness depending on the developmental stage in which these selective pressures occur. The selection protocol

used in this study selectively applied nutrient shortages during adulthood only, limiting selection pressures to traits that enhance adult starvation resistance. However, we found that starvation selection increases body size as early as the second instar stage and persists throughout the rest of development. Similarly, we identified larval-specific effects on food consumption and sleep. We found that food intake is increased in larvae and reduced in adults, and that sleep is reduced in adults but unchanged in larvae. Our sleep analysis was limited to second instar larvae, therefore we were unable to determine whether the feeding and metabolic phenotypes observed in third instar larvae also extend to sleep. To date, sleep characterization is limited to second instar larvae, and technical challenges, including the size and mobility of third instar larvae provide technical impediments to sleep analysis at this developmental stage. However, our findings in second instar larvae provide further evidence that selection for starvation resistance results in ontogenetically specified behavioral phenotypes. It has been previously shown that selective stresses imposed during development contribute to altered behavioral states as adults. In several *Drosophila* species, for example, thermal stress applied during larval development confers resistance to thermal stress in adulthood (Levins, 1969; Goto, 2000; Horu and Kimuro, 1998; Maynard Smith, 2005). Therefore, selection for starvation resistance during a defined developmental window can impact a variety of traits at multiple stages throughout development.

Although we found that changes in development rate, metabolic function and sleep differ in starvation-selected populations, the genetic contribution of each trait to starvation resistance, especially at each stage of development, is unknown. We observed increased sleep and decreased starvation-induced feeding in starvation-selected adults, traits that are not present during larval development. Although these traits provide a potential mechanism for energy conservation in adult flies, in larvae, development rate slows, sleep does not differ and food consumption actually increases. Although food intake was increased in starvation-selected larvae, their feeding rate remained unchanged. It is possible, although not assessed in this study, that this may be a result of decreased activity. However, it is likely that this increase is related to their larger body size and results from increased food intake per mouth hook contraction. This, together with our findings that metabolic rate is reduced in both larvae and adults of starvation-selected populations, supports a model by which a slower development provides increased time to grow and accumulate energy stores as larvae, while reducing foraging-related behaviors in adulthood allows for animals to conserve energy as adults when food is not present during the selection process. This model suggests that distinct genetic architecture regulates sleep and feeding during the larval and adult stages. For instance, the mechanisms controlling larval sleep are partially distinct from that of adult sleep (Szuperak et al., 2018). Overall, these findings provide proof of principle for ontogeny-specific correlated behaviors during the applied starvation-selection process.

The identification of developmental, metabolic and behavioral differences in *Drosophila* populations selected for resistance to starvation suggest that multiple mechanisms likely contribute to the etiology of starvation resistance. Towards this end, association mapping in *Drosophila* identified a wide range of genes associated with starvation resistance, including those that are known regulators of development, metabolism and nutrient response (Harbison et al., 2004; Nelson et al., 2016). The complex genetic architecture underlying these traits, and their inter-relationship, suggests the evolution of starvation resistance is likely to be highly pleiotropic. A previous study examining the genetic divergence between these populations identified 1796 polymorphisms that significantly differed between starvation-selected and control populations. These polymorphisms mapped to a set of 382 genes, including genes associated with a wide variety of metabolic and physiological processes (Hardy et al., 2018). Indeed, there is experimental evidence for this in a naturally occurring population of *Drosophila*, in which a seasonally fluctuating molecular polymorphism in the *Insulin-like receptor* (*InR*) has been linked to increased starvation resistance, decreased fecundity and increased lifespan (Paaby et al., 2014). Given that we observed population-specific differences in mass, metabolic rate and sleep, it is also possible that distinct mechanisms contribute to starvation resistance in each of the three replicate populations. As such, genomic sequencing of these populations revealed a low correlation of allele frequencies between the starvation-selected populations, suggesting that there are indeed multiple mechanisms of adaptation. While these studies provide an initial framework for identifying genetic factors regulating traits contributing to starvation resistance, a typical limitation of studying selected populations is a lack of accessible genetic tools that can be applied to validate the phenotypic contributions of single genes. The recent application of gene-editing approaches to outbred and non-*Drosophila melanogaster* populations raises the possibility of examining the contributions of these candidate genes in the future. These findings reveal evidence of an ontogenetic shift associated with selection for starvation resistance in *Drosophila melanogaster*. This work highlights the contribution of several energy-saving traits that are modulated throughout development, including changes in metabolic rate, size, sleep and food consumption to confer resistance to starvation. The development-specific differences in sleep and feeding of the starvation-selected populations set the stage for elucidating the genetic basis of starvation resistance over the course of development.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: E.B.B., M.E.S., M.S., A.K., A.G., M.S.K., A.C.K.; Methodology: E.B.B., M.E.S., M.S., A.K., A.G.; Formal analysis: E.B.B., M.S., A.G.; Investigation: E.B.B., M.S., A.K., M.S.K., A.C.K.; Resources: M.S., M.S.K., A.C.K.; Writing - original draft: E.B.B., M.S.K., A.C.K.; Writing - review & editing: E.B.B., M.E.S., M.S., A.K., A.G., M.S.K., A.C.K.; Visualization: M.E.S., M.S., A.G.; Supervision: E.B.B., M.S.K., A.C.K.; Funding acquisition: A.G., M.S.K., A.C.K.

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Supplementary information
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References
Aggarwal, D. D. (2014). Physiological basis of starvation resistance in Drosophila leontia: analysis of sexual dimorphism. *J. Exp. Biol.* **217**, 1849-1859.

Baldal, E. A., Brakefield, P. M. and Zwaan, B. J. (2006). Multitrait evolution in lines of *Drosophila melanogaster* selected for increased starvation resistance: the role of metabolic rate and implications for the evolution of longevity. *Evolution (N. Y).* **60**, 1435-1444.

Blaxter, K. L. (1989). *Energy Metabolism in Animals and Man*. Cambridge University Press.

Bubliy, O. A. and Loeschcke, V. (2005). Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. *J. Evol. Biol.* **18**, 789-803.

Chippindale, A. K., Chu, T. J. F. and Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution (N. Y).* **50**, 753-766.

Church, R. B. and Robertson, F. W. (1966). Biochemical analysis of genetic differences in the growth of *Drosophila*. *Genet. Res.* **7**, 383-407.
Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J. and Léopold, P. (2003). A nutrient sensor mechanism controls Drosophila growth. *Cell* **114**, 739-749.
Djawdan, M., Rose, M. R. and Bradley, T. J. (1997). Does selection for stress resistance lower metabolic rate? *Ecology* **78**, 828-837.
Dulloo, A. G. and Jacquet, J. (1998). Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. *Am. J. Clin. Nutr.* **68**, 599-606.
Ewing, A. W. (1961). Body size and courtship behavior in *Drosophila melanogaster*. *Anim. Behav.* **9**, 93-99.
Featherstone, D. E., Chen, K. and Broadie, K. (2009). Harvesting and preparing *Drosophila* embryos for electrophysiological recording and other procedures. *J. Vis. Exp.* **27**, 1347.
Folguera, G., Ceballos, S., Spezzi, L., Fanara, J. J. and Hasson, E. (2008). Clinal variation in developmental time and viability, and the response to thermal treatments in two species of Drosophila. *Biol. J. Linn. Soc.* **95**, 233-245.
Garlapow, M. E., Everett, L. J., Zhou, S., Gearhart, A. W., Fay, K. A., Huang, W., Morozova, T. V., Arya, G. H., Turlapati, L., St, . et al. (2016). Genetic and genomic response to selection for food consumption in *Drosophila melanogaster*. *Behav. Genet.* **47**, 227-243.
Gefen, E., Marlon, A. J. Gibbs, A. G. (2006). Selection for desiccation resistance in adult *Drosophila melanogaster* affects larval development and metabolite accumulation. *J. Exp. Biol.* **209**, 3293-3300.
Gibbs, A. G. and Reynolds, L. A. (2012). Drosophila as a model for starvation: Evolution, physiology, and genetics. In *Comparative Physiology of Fasting, Starvation, and Food Limitation* (ed. M. McCue), pp. 37-51. Springer.
Goto, S. G. (2000). Expression of Drosophila homologue of senescence marker protein-30 during cold acclimation. *J. Insect. Physiol.* 46, 1111-1120.
Harbison, S. T., Yamamoto, A. H., Fanara, J. J., Norga, K. K. and Mackay, T. F. C. (2004). Quantitative trait loci affecting starvation resistance in *Drosophila melanogaster*. *Genetics* **166**, 1807-1823.
Harbison, S. T., Serrano Negron, Y. L., Hansen, N. F. and Lobell, A. S. (2017). Selection for long and short sleep duration in *Drosophila melanogaster* reveals the complex genetic network underlying natural variation in sleep. *PLoS Genet.* **13**, e1007098.
Hardy, C. M., Burke, M. K., Everett, L. J., Han, M. V., Lantz, K. M. and Gibbs, A. G. (2018). Genome-wide analysis of starvation-selected *Drosophila melanogaster*—a genetic model of obesity. *Mol. Biol. Evol.* **35**, 50-65.
Harshman, L. G. and Schmid, J. L. (1998). Evolution of starvation resistance in Drosophila melanogaster: aspects of metabolism and counter-impact selection. *Evolution (N. Y).* **52**, 1679-1685.
Hendricks, J. C., Finn, S. M., Panckeri, K. A., Chavkin, J., Williams, J. A., Sehgal, A. and Pack, A. I. (2000). Rest in Drosophila is a sleep-like state. *Neuron* **25**, 129-138.
Hoffmann, A. A. and Harshman, L. G. (1999). Desiccation and starvation resistance in Drosophila: Patterns of variation at the species, population and intrapopulation levels. *Heredity (Edinb).* **83**, 637-643.
Hoffmann, A. A. and Parsons, P. A. (1989). An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in Drosophila. *Biol. J. Linn. Soc.* **37**, 117-136.
Horu, Y. and Kimuro, M. T. (1998). Relationship between cold stupor and cold tolerance in *Drosophila* (Diptera: Drosophilidae). *Environ. Entomol.* **27**, 1297-1302.
Ikeya, T., Galic, M., Belawat, P., Nairz, K. and Hafen, E. (2002). Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. *Curr. Biol.* **12**, 1293-1300.
Itskov, P. M. and Ribeiro, C. (2013). The dilemmas of the gourmet fly: The molecular and neuronal mechanisms of feeding and nutrient decision making in Drosophila. *Front. Neurosci.* **7**, 1-13.
Kaun, K. R., Riedl, C. A. L., Chakaborty-Chatterjee, M., Belay, A. T., Douglas, S. J., Gibbs, A. G. and Sokolowski, M. B. (2007). Natural variation in food acquisition mediated via a Drosophila cGMP-dependent protein kinase. *J. Exp. Biol.* **210**, 3547-3558.
Koh, K., Evans, J. M., Hendricks, J. C. and Sehgal, A. (2006). A Drosophila model for age-associated changes in sleep:wake cycles. *Proc. Natl. Acad. Sci. USA* **103**, 13843-13847.
Kolss, M., Vijendravarma, R. K., Schwaller, G. and Kawecki, T. J. (2009). Life-history consequences of adaptation to larval nutritional stress in drosophila. *Evolution.* **63**, 2389-2401.
Layalle, S., Arquier, N. and Léopold, P. (2008). The TOR pathway couples nutrition and developmental timing in Drosophila. *Dev. Cell* **15**, 568-577.
Levins, R. (1969). Thermal Acclimation and Heat Resistance in *Drosophila* species. *Am. Naturalist* **103**, 483-499.
Liu, S., Li, K., Gao, Y., Liu, X., Chen, W., Ge, W., Feng, Q., Palli, S. R. and Li, S. (2017). Antagonistic actions of juvenile hormone and 20-hydroxyecdysone within the ring gland determine developmental transitions in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **115**, 139-144.
Ma, S. W. Y. and Foster, D. O. (1986). Starvation-induced changes in metabolic rate, blood flow, and regional energy expenditure in rats. *Can. J. Physiol. Pharmacol.* **64**, 1252-1258.
Marron, M. T., Markow, T. A., Kain, K. J. and Gibbs, A. G. (2003). Effects of starvation and desiccation on energy metabolism in desert and mesic Drosophila. *J. Insect. Physiol.* **49**, 261-270.
Masek, P., Reynolds, L. A., Bollinger, W. L., Moody, C., Mehta, A., Murakami, K., Yoshizawa, M., Gibbs, A. G. and Keene, A. C. (2014). Altered regulation of sleep and feeding contribute to starvation resistance in Drosophila. *J. Exp. Biol.* **217**, 3122-3132.
Matzkin, L. M., Watts, T. D. and Markow, T. A. (2009). Evolution of stress resistance in Drosophila: interspecific variation in tolerance to desiccation and starvation. *Funct. Ecol.* **23**, 521-527.
Maynard Smith, J. (2005). Acclimatization to high temperatures in inbred and outbred Drosophila subobscura. J. Genet. 1956, 54, 497-505. *J. Genet.* **84**, 37-45.
McCue, M. D. (2010). Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **156**, 1-18.
Melcher, C. and Pankratz, M. J. (2005). Candidate gustatory interneurons modulating feeding behavior in the Drosophila brain. *PLoS Biol.* **3**, 1618-1629.
Nelson, C. S., Beck, J. N., Wilson, K. A., Pilcher, E. R., Kapahi, P. and Brem, R. B. (2016). Cross-phenotype association tests uncover genes mediating nutrient response in Drosophila. *BMC Genomics* **17**, 867.
Paaby, A. B., Bergland, A. O., Behrman, E. L. and Schmidt, P. S. (2014). A highly pleiotropic amino acid polymorphism in the Drosophila insulin receptor contributes to life-history adaptation. *Evolution (N.Y).* **68**, 3395-3409.
Pfeiffenberger, C., Lear, B. C., Keegan, K. P. and Allada, R. (2010). Locomotor activity level monitoring using the Drosophila activity monitoring (DAM) system. *Cold Spring Harb. Protoc.* **2010**, prot5518.
Pool, A.-H. and Scott, K. (2014). Feeding regulation in Drosophila. *Curr. Opin. Neurobiol.* **29**, 57-63.
Reynolds, L. A. (2013). The effects of starvation selection on *Drosophila melanogaster* life history and development. PhD thesis, University of Nevada, Las Vegas.
Riddiford, L. M. (1993). Hormones and *Drosophila* development. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 899-939. Cold Spring Harbor Laboratory Press.
Rion, S. and Kawecki, T. J. (2007). Evolutionary biology of starvation resistance: what we have learned from Drosophila. *J. Evol. Biol.* **20**, 1655-1664.
Rose, M. R., Nusbaum, T. J. and Chippindale, A. K. (1996). Laboratory evolution: The experimental wonderland and the Cheshire cat syndrome. In *Adaptation* (ed. M. R. Roe and G. V. Lauder), pp. 221-241. San Diego: Academic Press.
Schmidt, M. H. (2014). The energy allocation function of sleep: a unifying theory of sleep, torpor, and continuous wakefulness. *Neurosci. Biobehav. Rev.* **47**, 122-153.
Schwasinger-Schmidt, T. E., Kachman, S. D. and Harshman, L. G. (2012). Evolution of starvation resistance in Drosophila melanogaster: Measurement of direct and correlated responses to artificial selection. *J. Evol. Biol.* **25**, 378-387.
Shaw, P. J., Cirelli, C., Greenspan, R. J. and Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. *Science* **287**, 1834-1837.
Shen, P. (2012). Analysis of feeding behavior of drosophila larvae on liquid food. *Cold Spring Harb. Protoc.* **7**, 568-571.
Slaidina, M., Delanoue, R., Gronke, S., Partridge, L. and Léopold, P. (2009). A drosophila insulin-like peptide promotes growth during nonfeeding states. *Dev. Cell* **17**, 874-884.
Slocumb, M. E., Regalado, J. M., Yoshizawa, M., Neely, G. G., Masek, P., Gibbs, A. G. and Keene, A. C. (2015). Enhanced sleep is an evolutionarily adaptive response to starvation stress in Drosophila. *PLoS ONE* **10**, 1-16.
Stahl, B., Slocumb, M., Chaitin, H., DiAngelo, J. and Keene, A. (2017). Sleep-dependent modulation of metabolic rate in Drosophila. *Sleep* **40**, zsx084.
Sternson, S. M., Nicholas Betley, J. and Cao, Z. F. H. (2013). Neural circuits and motivational processes for hunger. *Curr. Opin. Neurobiol.* **23**, 353-360.
Svetec, N., Zhao, L., Saelao, P., Chiu, J. C. and Begun, D. J. (2015). Evidence that natural selection maintains genetic variation for sleep in Drosophila melanogaster. *BMC Evol. Biol.* **15**, 41.
Szuperak, M., Churgin, M. A., Borja, A. J., Raizen, D. M., Fang-Yen, C. and Kayser, M. S. (2018). A sleep state in Drosophila larvae required for neural stem cell proliferation. *Elife* **7**, e33220.
Tennessen, J. M. and Thummel, C. S. (2011). Coordinating growth and maturation - Insights from Drosophila. *Curr. Biol.* **21**, R750-R757.
Wang, T., Hung, C. C. Y. and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Annu. Rev. Physiol.* **68**, 223-251.
Wayne, M. L., Soundararajan, U. and Harshman, L. G. (2006). Environmental stress and reproduction in Drosophila melanogaster: Starvation resistance, ovariole numbers and early age egg production. *BMC Evol. Biol.* **6**, 1-10.
Wong, R., Piper, M. D. W., Wertheim, B. and Partridge, L. (2009). Quantification of food intake in Drosophila. *PLoS One* **4**, e6063.
Yadav, P. and Sharma, V. K. (2014). Correlated changes in life history traits in response to selection for faster pre-adult development in the fruit fly Drosophila melanogaster. *J. Exp. Biol.* **217**, 580-589.
Yamanaka, N., Rewitz, K. F. and O'Connor, M. B. (2013). Ecdysone control of developmental transitions: lessons from *Drosophila* research. *Annu. Rev. Entomol.* **58**, 497-516.
Yurgel, M., Masek, P., DiAngelo, J. R. and Keene, A. (2014). Genetic dissection of sleep-metabolism interactions in the fruit fly. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **201**, 869-877.

FIG. 1. SURVIVORSHIP

A ADULT

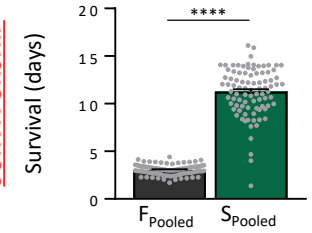
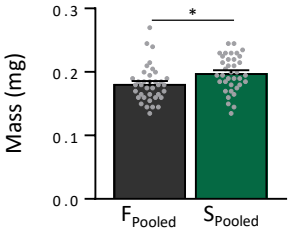
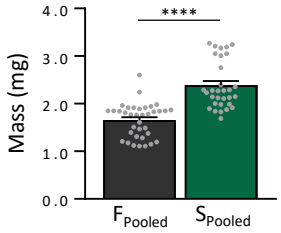


FIG. 1. MASS

B 2nd Instar



C 3rd Instar



D Adult

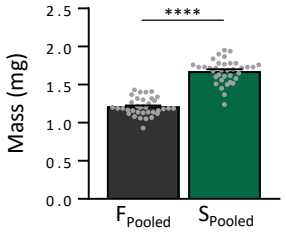
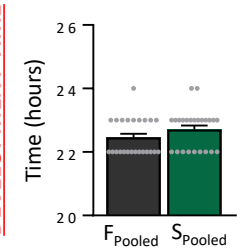
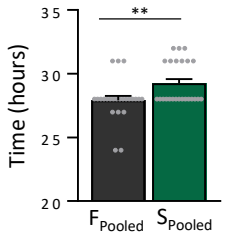


FIG. 2. DEVELOPMENT TIME

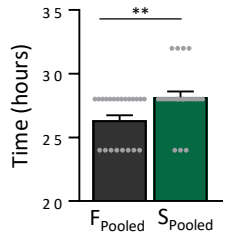
E Egg – 1st Instar



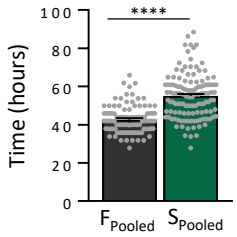
F 1st – 2nd Instar



G 2nd – 3rd Instar



H 3rd Instar - Pupariation



I Pupariation to Eclosion

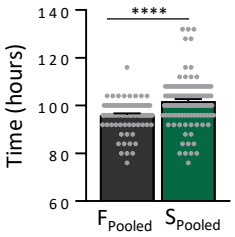
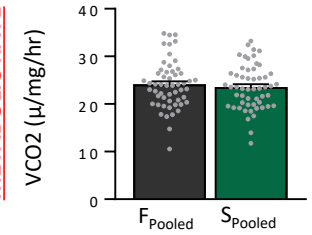
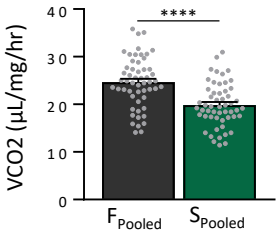


FIG. 3. METABOLIC RATE

J 2nd Instar



K 3rd Instar



L Adult

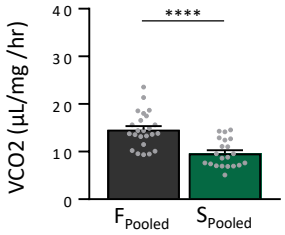
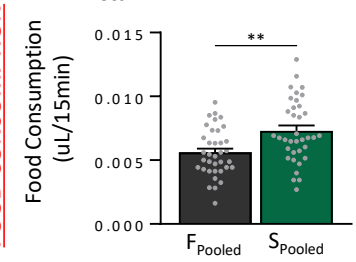
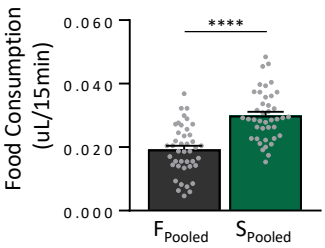


FIG. 4. FOOD CONSUMPTION

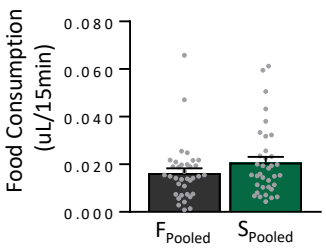
M 2nd Instar



N 3rd Instar



O Adult - Fed



P Adult - Starved

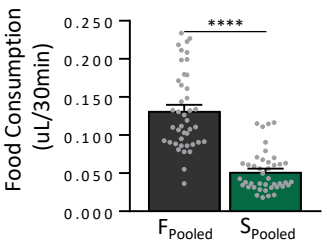
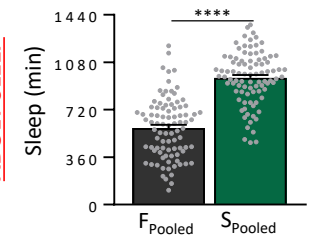
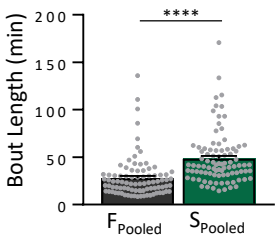


FIG. 5. ADULT SLEEP

Q Adult



R



S

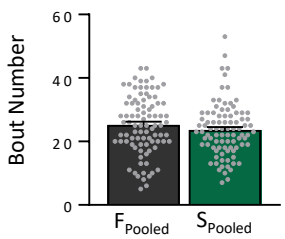
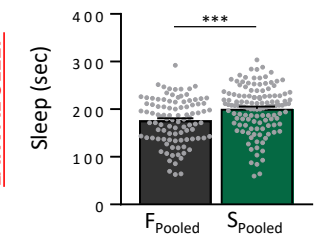
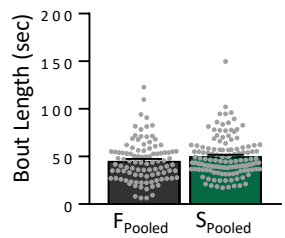


FIG. 5. LARVAL SLEEP

T 2nd Instar



U



V

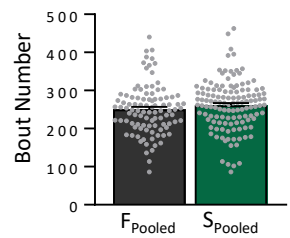


Fig. S1. Pooled means of the control (F_{Pooled}) and starvation-selected (S_{Pooled}) populations for each trait displayed in the primary text. Data shown include: survivorship and mass (row 1); development time (row 2); metabolic rate (row 3); Food consumption (row 4); adult sleep (row 5); and larval sleep (row 6). Stars indicate instances where the two-way ANOVA model referenced in the primary text reached significance. * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; **** = $P<0.0001$. Error bars represent +/- standard deviation from mean.

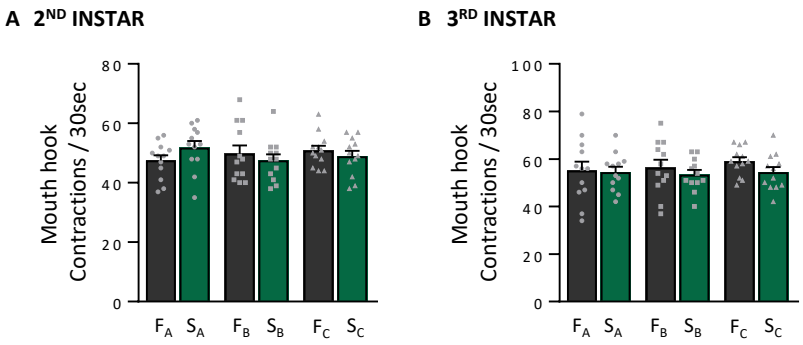


Fig. S2. Increased food consumption in starvation resistant larvae is not a result of changes in feeding rate. There is no difference in the number of mouth hook contractions taken during a 30 sec period in either (A) 2nd instar larvae (two-way ANOVA: $F_{1,66} = 0.0003$, P=0.9870, N = 12 per population) or (B) 3rd instar larvae (two-way ANOVA: $F_{1,66} = 1.569$, P=0.2148, N = 12 per population). Error bars represent +/- standard deviation from mean.