Chronic malnutrition favours smaller critical size for metamorphosis initiation in *Drosophila melanogaster*

Roshan K. Vijendravarma, Sunitha Narasimha, Tadeusz J. Kawecki

Department of Ecology and Evolution, University of Lausanne, Switzerland

Correspondence: tadeusz.kawecki@unil.ch

Journal of Evolutionary Biology 25:288-292 (2012) doi: 10.1111/j.1420-9101.2011.02419.x

Except of small corrections in proofs, the content of this preprint is identical with the published version.

Keywords: experimental evolution, stress tolerance, dietary restriction, critical weight, life history, trade-offs, starvation resistance

Abstract

Critical size at which metamorphosis is initiated represents an important checkpoint in insect development. Here we use experimental evolution in *Drosophila melanogaster* to test the long-standing hypothesis that larval malnutrition should favour a smaller critical size. We report that six fly populations subject to 112 generations of laboratory natural selection on an extremely poor larval food evolved an 18 % smaller critical size (compared to six unselected control populations). Thus, even though critical size is not plastic with respect to nutrition, smaller critical size can evolve as an adaptation to nutritional stress. We also demonstrate that this reduction in critical size (rather than differences in growth rate) mediates a trade-off in body weight that the selected populations experience on standard food, on which they show a 15-17 % smaller adult body weight. This illustrates how developmental mechanisms which control life history may shape constraints and trade-offs in life history evolution.

Introduction

Life history traits such as age and size at maturity often show remarkable plasticity and evolvability. In anatomically complex metazoans, plastic and evolutionary shifts in life history must be precisely regulated to ensure synchronized and coordinated development across body parts, organs, and tissues, irrespective of the environmental and genetic causes of the shift. This regulation relies on endocrine or neural signals which are emitted centrally and modulate cell growth, proliferation and differentiation throughout the body. However, it must also involve checkpoints and feedbacks that keep the neuroendocrine system “informed” about the state of the organism and its parts. Comprehensive understanding of life history evolution requires understanding how these signals and checkpoints evolve under particular environmental conditions.

Here we focus on one such checkpoint in the development of holometabolous insects: the critical size at which metamorphosis is initiated. Although experimentally measured in terms of larval weight, the assessment of critical size during development (at least in *Drosophila*) seems to rely on stretch receptors in the neuroendocrine organ named the ring gland (Mirth et al., 2005). Once the critical size is reached, a

pulse of ecdysone secretion commits the larva to metamorphosis. Even though feeding continues for some time and more than half of the final mass may be gained after the critical size, the interval between reaching critical size and pupation is not affected by nutrition; the larva will attempt pupation even if completely deprived of food (De Moed et al., 1999; Edgar, 2006). The critical size is likewise not affected by nutrition (De Moed et al., 1999; Mirth et al., 2005; Edgar, 2006). As a consequence, the plastic response of adult size to nutrition reflects the growth occurring after the critical size whereas variation in developmental time mostly reflects the time needed to reach critical size.

Drosophila larvae show a remarkable capacity to compensate for poor nutrition and slow growth by more than doubling time to pupation, with only mild increase in intrinsic mortality (e.g., Gebhardt & Stearns, 1993; Kolss et al., 2009). However, this plasticity may be too costly if predation is high or the food resource is ephemeral or deteriorates with time. It has thus been proposed that larval malnutrition should favour small critical size, even if this comes at the cost of smaller adult size and thus lower fertility or mating success (Royes & Robertson, 1964; De Moed et al., 1999). Consistent with this hypothesis, although *Drosophila funebris* and *D. immigrans* have similar adult size, the former is much more tolerant to poor larval food – and it has 35 % smaller critical weight (Royes & Robertson, 1964). Yet, several hundred generations of experimental evolution under larval crowding failed to change the critical size in *D. melanogaster* (Santos et al., 1997), even though other studies demonstrated genetic variance for this trait (De Moed et al., 1999; Partridge et al., 1999).

We address this hypothesis using six populations of *D. melanogaster* maintained on poor (i.e., diluted) larval food for 112 generations and simultaneously subject to selection for relatively fast development; six control populations have been maintained in parallel on standard food (Kolss et al., 2009; Vijendravarma et al., 2011). With about 0.3 % yeast concentration and less than 150 calories per ml, the poor food exerts extreme nutritional stress on the larvae. Raised on the poor food, flies from unselected populations emerge at less than half of normal body weight despite twice as long larval development time (Kolss et al., 2009; Vijendravarma et al., 2011). Adaptation to poor food in the experimental populations is manifested in their improved viability, faster growth and shorter developmental time when raised on poor food. However, correlated responses to selection include a 15-17 % lower adult weight and 20 % lower fecundity (compared to control populations) in flies raised on standard food (Kolss et al., 2009; Vijendravarma et al., 2011). Here we test whether the selected populations evolved a smaller critical size, and whether this smaller critical size (rather than differences in growth rate) mediates the body size trade-off observed on the standard food.

Materials and methods

Experimental evolution. Twelve populations were derived from a single outbred laboratory population (Kolss et al., 2009). Six populations have been subject to 112 generations of laboratory natural selection for tolerance to chronic larval malnutrition by being maintained on poor larval food; six control populations have been maintained on standard food. The standard food consists of 15 g agar, 12.5 g dry yeast, 30 g sucrose, 60 g glucose, 50 g cornmeal, 0.5 g $MgSO_4$, 0.5 g $CaCl_2$, 30 ml ethanol, 6 ml propionic acid, and 1 g nipagin per litre of water; the poor food contains ¼ as much yeast, sugars and cornmeal as the standard food. (Vijendravarma et al., 2011). Larval density was low (maintained at 200 eggs/30 ml of food), corresponding to 22 calories available per larva on poor food, ten times the energy content of a large pre-pupation larva (Vijendravarma et al., 2011). Only adults that emerged within 14 days of oviposition were used to breed the next generation (sometimes this cut-off had to be relaxed to ensure enough adults), resulting in additional selection against delayed development on poor food. Adults (census number about 150 individuals) were fed standard food with live yeast supplement.

Critical size and pre-critical growth. Critical size is estimated as the weight which, once attained, allows half of the larvae to pupariate upon subsequent starvation (De Moed et al., 1999; Mirth et al., 2005). Before the assay all populations were reared for two generations on standard food. Prior to egg collection the parents were allowed to mate and oviposit for 48 h to avoid egg retention. Fourteen vials with 30 ml of standard food were each seeded with 200 eggs (collected over 2 h) and incubated at 25ºC and 70 % humidity under constant light. At 8 h intervals between 84 and 132 h after oviposition larvae were collected from two random vials per population (with about 22 h from oviposition to egg hatching, this corresponds to about 62 to 110 h post-hatching). A group of five randomly picked larvae were dried on a filter paper for few seconds and weighed to the nearest microgram, while 20 other random larvae from the same sample were transferred to a vial containing 2 % agar (food deprived) and incubated at the same conditions. The number of those larvae that subsequently formed puparia was scored.

We used two alternative estimates of critical weight. First, for each replicate population we fit a logistic regression of proportion of larvae forming puparia (p) on log weight at food deprivation (pooled across the two replicate vials), and estimated log critical weight as the point where this regression line crosses 0.5 (i.e., – intercept/slope). However, about 5 % of larvae did not pupariate even when starved after 132 h of feeding (see Results). If this 5 % is an estimate of the fraction of larvae that fail to pupariate despite reaching critical weight, we can account for this by fitting a modified logistic model [2]

$$\ln(p/(0.95 - p)) = a + b \ln(\text{weight}), \quad (1)$$

and calculating an alternative estimate of the log(critical weight) as $-a/b$, corresponding to the weight which upon starvation results in half of the maximum pupariation success (i.e., 0.95/2). Both estimates were compared between selection regimes with a t-test.

We fit a general mixed model (GMM) to log transformed larval weights to compare larval growth trajectories, with larval age as a continuous variable, selection regime as a fixed factor, population as a random factor nested within selection regime, and regime×age and population×age interactions.

Results

None of the larvae removed from food at 84 or 92 h formed puparia (Fig. 1a). The proportion of larvae removed from food at 132 h that successfully pupariated did not differ between selection regimes (mean ± SE: selected 0.962 ± 0.011, control, 0.946 ± 0.015, t_{10} = 0.9, P = 0.39). In between, the proportion of larvae forming puparia generally increased with the length of time they were allowed to feed (and thus with weight at food deprivation). An exception is the pupariation success of larvae deprived of food 100 h post-oviposition, which, for unknown reasons, for both control and selected populations was as high as that of larvae deprived at 108 h. At all intermediate time points (100-124 h) the estimated mean proportion of larvae forming puparia was greater for the selected than control population (Fig. 1a).

Accordingly, the mean critical weight was about 17 % lower for selected than for control populations, irrespectively if it was estimated with standard logistic regression (0.48 ± 0.04 mg versus 0.58 ± 0.02 mg, t_{10} = 4.0, P = 0.0026), or with the modified logistic regression assuming 5 % weight-independent pupariation failure (0.45 ± 0.03 mg versus 0.56 ± 0.02 mg, t_{10} = 5.0, P = 0.0005). To check the robustness of these results to possible problems due to the higher-than-expected pupariation success of larvae deprived of food at 100 h (see above), we also estimated the critical weights with the 100 h data removed. This had negligible effect on the mean critical weight estimates (0.46 ± 0.04 mg versus 0.56 ± 0.02 mg, t_{10} = 4.1, P = 0.0020). For all methods of estimation, the estimates of critical weight for the two sets of populations did not overlap (Fig. 1b).

At 124 and 132 h the larvae of the selected populations tended to be smaller (Fig. 2); however by this time many larvae from the selected populations had already entered the wandering stage, and by 132 h some

had even pupariated. Analysis of growth trajectories prior to critical weight (i.e., based on larvae weighed at 84 to 116 h, Fig. 2) revealed no difference between the selection regimes (GMM, regime $F_{1,10}$ = 0.1, P = 0.79; regime×age $F_{1,10}$ = 1.9, P = 0.20).

Discussion

Our results support the hypothesis that poor larval diets favour smaller critical size for metamorphosis initiation, especially if the resource deteriorates or is ephemeral. Our selection regime favoured the ability to survive, grow and complete development on the poor food within limited time and still be fertile as adult. Based on our data, we cannot say to what extent the shift in critical size is due to selection for fast development imposed by the cut-off on emergence time irrespective of food quality. However, in another study, fly populations selected for fast development on good food did not show reduction in critical size; rather, they achieved faster development through faster pre-critical growth (Prasad et al., 2001). Likewise, experimental evolution under larval crowding did not affect critical size (Santos et al., 1997). Under the larval crowding regime, the food quality is initially excellent and then deteriorates rapidly as the food is being consumed and contaminated with waste products. It thus presumably favours larvae which can take quick advantage of the initial prosperity, even the expense of poorer resource use efficiency. Consistent with this, larvae of populations adapted to crowding are fast eaters and fast growers, but show a poor efficiency of converting larval into adult biomass (Santos et al., 1997). In contrast, under our selection regime, the nutritional environment is poor from the start, and deteriorates further only slowly because larval density is low. Growth under these conditions is likely to be limited at least in part by metabolic efficiency, so high efficiency is likely to be favoured. In contrast, under rich food conditions, larval growth rate is more likely to be limited by the rate at which cells can divide and differentiate. Consistent with this, larvae of our selected populations grow faster than controls on the poor food (Kolss et al., 2009), but not on the standard food (this study). Although other reasons (e.g., genetic background, experimental details) cannot be excluded, these differences among studies suggest that the evolution of smaller critical size in our study is specifically linked to larval malnutrition. Interestingly, selection for small adult size also leads to smaller critical size with no change in developmental time (Partridge et al., 1999). One can thus speculate that smaller critical size is favoured mostly because it allows smaller larvae to complete development rather than because it accelerates development.

The results also support our prediction that the reduction in critical size largely mediates the trade-off in adult body size expressed on the standard food, where the selected lines emerge at a 15-17 % smaller adult size while their larval development is only about 12-14 h shorter (Kolss et al., 2009; R. K. Vijendravarma, unpublished data). Prior to reaching critical size, both selected and control larvae grow with the same rate. Larval growth until the larvae cease feeding is to a good approximation exponential (e.g. Partridge et al., 1999). Under exponential growth at rate b, the ratio of weights taken at two ages Δt apart equals $\exp(-b\Delta t)$. With a constant post-critical feeding time, Δt between the ages at which selected and control populations reach critical size would be the same as Δt between the ages at which they cease feeding. Thus, the ratio of the critical weight between the selected and control populations should predict the ratio between their weights when they stop feeding. Assuming the same conversion of larval to adult weight, the latter ratio should in turn predict the ratio of adult weights. It follows that, other things being equal, the ratio of critical weights of selected to control populations should predict the ratio of adult weights. This prediction is well borne out – the estimates of these ratios are 0.82-0.83 for critical weight (depending on which of the two measures are used), 0.83 for adult female weight and 0.85 for male weight (Vijendravarma et al., 2011). Even though some contribution of post-critical growth rate or duration to differences in adult weight cannot be excluded, it is parsimonious to conclude that the smaller adult size of selected flies raised on standard food is largely a consequence of smaller critical size.

In contrast, the smaller critical size alone cannot explain the difference in adult size when larvae are raised on poor food – here the selected larvae grow faster (Kolss et al., 2009), so that they are only slightly smaller as adults (2 % females, 5 % males; Vijendravarma et al., 2011), even though they take 3.5 days less to develop. However, without a change in critical size, faster post-critical growth would lead to a larger adult size, so the smaller critical size allowed selected populations to “convert” their faster growth to a much shorter development, with no increase but rather a slight decrease in adult size.

Our results may also explain the contrasting cellular basis of plastic versus evolutionary changes in wing size, which parallel changes in adult weight (Vijendravarma et al., 2011). Raised on standard food, selected flies have smaller wings than controls, owing entirely to reduced cell number (Vijendravarma et al., 2011). This is consistent with the fact that cell proliferation in the wing imaginal discs slows markedly down upon reaching the critical size (Neto-Silva et al., 2009), which occurs earlier in the selected lines. In contrast, plasticity of wing size in response to larval food is mostly mediated by cell size, presumably reflecting slower post-critical cell growth on the poor food (Vijendravarma et al., 2011).

This study demonstrates that larval critical size can evolve in response to an environment with persistent nutritional stress. Furthermore, because of its central role in insect development and its lack of plastic response to nutrition, the critical size will also mediate correlated responses expressed in other environments.

Acknowledgements

We thank B. Hollis and three reviewers for comments. This work was supported by the Swiss National Science Foundation.

References

De Moed, G. H., Kruitwagen, C., De Jong, G. & Scharloo, W. 1999. Critical weight for the induction of pupariation in Drosophila melanogaster: genetic and environmental variation. *Journal of Evolutionary Biology* **12**: 852-858.

Edgar, B. A. 2006. How flies get their size: genetics meets physiology. *Nature Reviews Genetics* **7**: 907-916.

Gebhardt, M. D. & Stearns, S. C. 1993. Phenotypic plasticity for life history traits in *Drosophila melanogaster*. I. Effect on phenotypic and environmental correlations. *Journal of Evolutionary Biology* **6**: 1-16.

Kolss, M., Vijendravarma, R. K., Schwaller, G. & Kawecki, T. J. 2009. Life history consequences of adaptation to larval nutritional stress in *Drosophila*. *Evolution* **63**: 2389–2401.

Mirth, C., Truman, J. W. & Riddiford, L. M. 2005. The role of the prothoracic gland in determining critical weight for metamorphosis in *Drosophila melanogaster*. *Current Biology* **15**: 1796-1807.

Neto-Silva, R. M., Wells, B. S. & Johnston, L. A. 2009. Mechanisms of growth and homeostasis in the *Drosophila* wing. *Annual Review of Cell and Developmental Biology* **25**: 197-220.

Partridge, L., Langelan, R., Fowler, K., Zwaan, B. & French, V. 1999. Correlated responses to selection on body size in *Drosophila melanogaster*. *Genetical Research* **74**: 43-54.

Prasad, N. G., Shakarad, M., Anitha, D., Rajamani, M. & Joshi, A. 2001. Correlated responses to selection for faster development and early reproduction in *Drosophila*: The evolution of larval traits. *Evolution* **55**: 1363-1372.

Royes, V. W. & Robertson, F. W. 1964. The nutritional requirements and growth relations of different species of *Drosophila*. *Journal of Experimental Zoology* **156**: 105-136.

Santos, M., Borash, D. J., Joshi, A., Bounlutay, N. & Mueller, L. D. 1997. Density-dependent natural selection in *Drosophila*: evolution of growth rate and body size. *Evolution* **51**: 420-432.

Vijendravarma, R. K., Narasimha, S. & Kawecki, T. J. 2011. Plastic and evolutionary responses of cell size and number to larval malnutrition in *Drosophila melanogaster*. *Journal of Evolutionary Biology* **24**: 897–903.

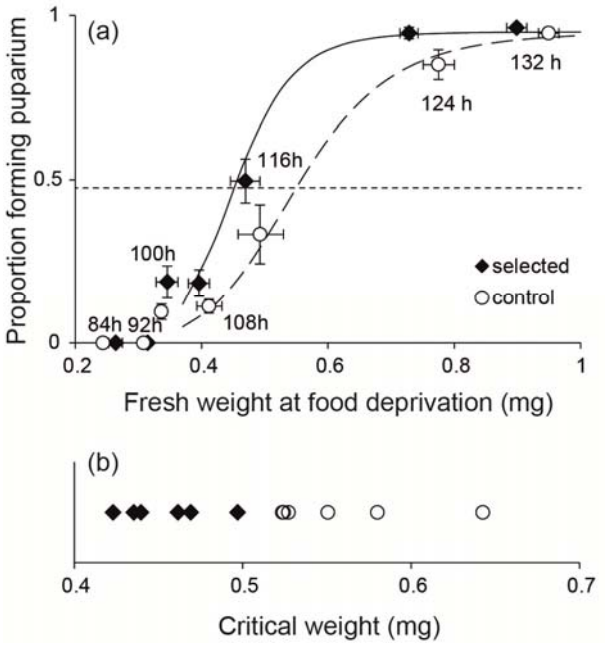

Figure 1. (a) Proportion of larvae forming puparium as a function of their average fresh weight at the onset of food deprivation; numbers indicate their age at food deprivation (from oviposition, i.e., including time to egg hatching). The symbols show means of the two selection regimes, the error bars correspond to standard errors based on variation among replicate populations within selection regimes. The curves were fitted with modified logistic regression (eqn 1), the dotted line corresponds to half of the maximum pupariation success (see Material and Methods). (b) Point estimates of critical weight for each selected and control population based on fitting equation (1).

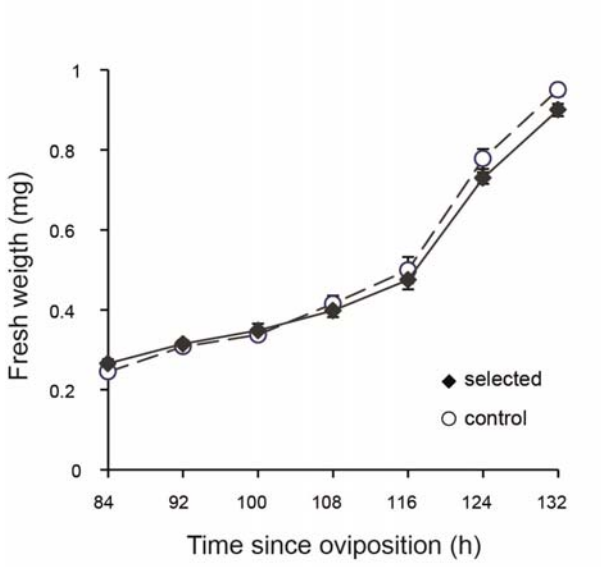

Figure 2. The mean growth trajectories of the selected and control populations. The bars correspond to ± one standard error. The underlying data are the same as those in Fig. 1a.