Temperature dependent larval resource allocation shaping adult body size in *Drosophila melanogaster*

Z. BOCHDANOVITS & G. DE JONG
Evolutionary Population Biology, Utrecht University, Padualaan, Utrecht, The Netherlands

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- geographical cline;
- phenotypic plasticity;
- resource allocation;
- size;
- temperature.

**Abstract**
Geographical variation in *Drosophila melanogaster* body size is a long-standing problem of life-history evolution. Adaptation to a cold climate invariably produces large individuals, whereas evolution in tropical regions result in small individuals. The proximate mechanism was suggested to involve thermal evolution of resource processing by the developing larvae. In this study an attempt is made to merge proximate explanations, featuring temperature sensitivity of larval resource processing, and ultimate approaches focusing on adult and pre-adult life-history traits. To address the issue of temperature dependent resource allocation to adult size vs. larval survival, feeding was stopped at several stages during the larval development. Under these conditions of food deprivation, two temperate and two tropical populations reared at high and low temperatures produced different adult body sizes coinciding with different probabilities to reach the adult stage. In all cases a phenotypic trade-off between larval survival and adult body size was observed. However, the underlying pattern of larval resource allocation differed between the geographical populations. In the temperate populations larval age but not weight predicted survival. Temperate larvae did not invest accumulated resources in survival, instead they preserved larval biomass to benefit adult weight. In other words, larvae from temperate populations failed to re-allocate accumulated resources to facilitate their survival. A low percentage of the larvae survived to adulthood but produced relatively large flies. Conversely, in tropical populations larval weight but not age determined the probability to reach adulthood. Tropical larvae did not invest in adult size, but facilitated their own survival. Most larvae succeeded in pupating but then produced small adults. The underlying physiological mechanism seemed to be an evolved difference in the accessibility of glycogen reserves as a result of thermal adaptation. At low rearing temperatures and in the temperate populations, glycogen levels tended to correlate positively with adult size but negatively with pupation probability. The data presented here offer an explanation of geographical variation in body size by showing that thermal evolution of resource allocation, specifically the ability to access glycogen storage, is the proximate mechanism responsible for the life-history trade-off between larval survival and adult size.

**Introduction**

**Thermal evolution of body size**
Temperature is almost certainly an important selective agent in shaping *Drosophila melanogaster* body size. Large body size has repeatedly been found to concur with a
cold evolutionary history (Huay et al., 1991). Geographical variation in D. melanogaster body size is also well documented; larger animals are found at higher latitudes (David et al., 1977; Coyne & Beecham, 1987; Gilchrist & Partridge, 1999).

A concise explanation for the thermal evolution of body size has, nevertheless, proved difficult to formulate. Larval growth and resource allocation is regarded as an important proximate determinant of variation in adult body size. Theoretical models predict that if a decrease of environmental temperature leads to a stronger decrease of the rate of processes consuming energy (i.e., metabolic rate) than of the growth rate, a larger adult can be realized (van der Have & De Jong, 1996; Atkinson & Sibly, 1997). Extension of this rationale to comparisons between populations and species is straightforward and has received empirical support, as temperature coefficients of growth rate and development rate have been shown to differ between Drosophila species (Gilbert & De Jong, 2001).

Empirical data support the notion that the evolution of resource processing by developing larvae is responsible for variation in adult body size. For a given amount of food and larval size, larger adults emerged in cold adapted laboratory lines (Neat et al., 1995; Robinson & Partridge, 2001). Larger body size at low temperature might be the result of increased resource allocation to the adult body mediated by the accumulation of higher mass-specific levels of glycogen in larvae (Z. Bochdanovits and G. de Jong, unpublished data).

In searching for an evolutionary explanation of geographical variation in body size, the most important issue is to determine what traits are under direct thermal selection. Evidence exists for evolved differences in both adult and larval fitness related traits. For both males and females it has been shown that larger animals have a higher fitness only at low temperatures. Artificial selection on body size, performed at 25 °C, resulted in large females that had higher fecundity and longevity but only at low rearing temperature (McCabe & Partridge, 1997). In a similar experiment it was shown that larger males have higher mating success only when tested at a low temperature (Reeve et al., 2000). Conversely, in another study male territorial success was highest when males reared at high temperature were competed at high temperature. Smaller adults reared at high temperatures were more successful at higher temperatures despite their smaller size (Zamudio et al., 1995). Moreover, early fecundity was higher when rearing temperature and test temperature were the same, supporting the idea of adaptive plasticity of body size (Nunney & Cheung, 1997). This evidence suggested a selective advantage for larger body size at lower temperatures, but not necessarily at higher temperatures.

Variation in pre-adult fitness related traits has been found in geographical populations and in laboratory selection lines maintained for years at high or low temperatures. Larval survival was higher at the rearing temperature corresponding to the evolutionary history of three of four geographical populations tested (Z. Bochdanovits and G. de Jong, unpublished data). In selection lines, larval competitive success was higher at the rearing temperature corresponding to their evolutionary history (Partridge et al., 1994; Partridge et al., 1995). These results indicate adaptive thermal evolution. However, the superior pre-adult survival of cold-adapted lines at low rearing temperatures diminished when larval crowding increased (Partridge et al., 1994; Partridge et al., 1995). At high larval density warm-adapted lines performed equally to the cold-adapted lines when tested at low rearing temperature. This finding suggested that the evolution of smaller body size at higher temperatures was accompanied by increased pre-adult competitive ability relative to the cold-adapted lines. An analysis of geographical variation in larval competitive ability performed on populations from an Australian north-south cline failed to support this hypothesis conclusively (James & Partridge, 1998). The role of competitive ability of larvae in temperature adaptation is not yet fully established; yet selection on pre-adult traits could well be of high importance for understanding the outcome of thermal evolution, including variation in adult body size.

Thermal evolution of resource allocation; a trade-off between size and survival

A major challenge in explaining adult body size remains to combine proximal explanations featuring resource allocation and ultimate explanations focusing on adult and pre-adult life history traits. The scenario that we propose here is that thermal evolution alters resource allocation to favour adult body size in cold environments and larval survival under warmer climates. At low temperatures, larval resource allocation might be tuned to favour investment in the future adult body if larger adult body size is advantageous and maintenance of larval survival does not require large investments. Evolution at cold temperatures would then result in preferential investment in adult body size by the developing larvae. Conversely, if at high temperatures investment in adult body size is not imperative although facilitating larval survival requires ongoing nutritional input, it could be expected that evolution at high temperature would result in preferential investment into traits ensuring maturation.

Our previous results indicated an evolved ability of temperate populations of D. melanogaster to accumulate higher mass-specific levels of resources and build larger adults at a given larval size (Z. Bochdanovits and G. de Jong, unpublished data). In the same study variation in larval survival was found. However, in that experiment the results might have been due to differences in resource acquisition between tropical and temperate populations. To investigate resource allocation per se, an
Experimental design needs to control for resource acquisition. Larvae at several stages of development are removed from their feeding medium and the resulting adult body size determined. This design allows estimating larval survival as the probability of larvae to pupate after being removed from the feeding medium prematurely. To prove the existence of a trade-off between adult body size and larval survival under these conditions, it is necessary to demonstrate that both traits depend on the allocation of the same limiting resource. Therefore, storage of metabolic reserves will be measured as well. The results will be discussed in the light of a possible explanation of the geographical variation of body size, in terms of evolved differential allocation of resources to larval and adult fitness associated traits.

Materials and methods

Populations

Wild living flies have been caught in Panama (8.5°N, 79.3°W; Barro Colorado), Congo (4.5°S, 11.5°E; Pointe Noire), Denmark (56.28°N, 9.25°E; Viborg) and the Netherlands (52.02°N, 5.1°E; Houten) between 6 and 12 months before the beginning of the experiment. Approximately one hundred females were collected from each of the sites. These populations had been cultured at large population sizes under constant light at 17.5 °C on a standard corn medium, prior to the beginning of the experiment.

Experimental conditions

Egg-laying females had been reared at their experimental temperatures, in order to control for possible maternal effects. Several hundred females oviposited for 3 h, at room temperature. Egg laying took place in empty jars covered with watch glasses containing 4 mL of a 1.9% agar-medium and a drop of a thick yeast suspension. Thirty eggs were put into a glass jar of height 5 cm and diameter 3.5 cm containing 15 mL of standard corn medium. From the eggs, larvae were reared at 17.5 °C and 27.5 °C. These temperatures are well within the physiological range that allows for normal development of D. melanogaster but represent its high and low regions. At several stages during development, when larvae were still feeding and normally would not yet pupariate, the larvae were removed from the standard corn medium. Development was interrupted at 45, 53, 61, 69, 81, 93, 102 h for the 27.5 °C group and at 80, 118, 130, 140, 154, 178, 200 h for the 17.5 °C group. These timepoints were chosen to represent a similar range of development at the different temperatures to allow for comparison across temperatures. They cover all of the three different larval stages: the final timepoint printed in bold represents the beginning of the prepupal stage, when undisturbed larvae start to pupariate. For statistical analysis, where data were pooled over the developmental temperatures, only the ages printed in bold were taken into account as they represent similar developmental stages (labelled as stage 1 through 5). For all populations, rearing temperatures and timepoints, larvae have been collected from eight replicate vials. Larvae collected from the eight replicate vials were pooled. Three groups of 10 larvae per timepoint were weighed, freeze-dried and stored at −30 °C for the biochemical assays. Five groups of 20 larvae were sorted for average size by eye, weighed and transferred to vials containing 2 mL agar medium for each timepoint. This procedure results in variation in larval weight between vials of the same stage. These larvae, which were prematurely deprived of further feeding, were allowed to pupate and the numbers of pupae were scored. The adults were collected 1 day after emergence and weighed. For collecting the larvae, a saturated sugar solution was poured on top of the medium, and the vials placed on an electrical heater. Between 15 and 45 min after adding the solution the floating larvae were collected from the replicate vials, using a paintbrush after pouring the solution thorough a sieve.

Measurements

Weight

Fresh and dry weight of larvae destined for the biochemical assays were determined in groups of 10, fresh and dry weight of larvae transferred to vials without food in groups of 20. Males and females were weighed per vial in the numbers as they emerged varying from one to 20. Fresh weight and dry weight were determined to the nearest 0.01 µg, using a Mettler ME 22 microbalance (Mettler-Toledo BV, Tiel, The Netherlands). For drying the specimens a Virtis 5 L freeze drier has been used.

Biochemical assays

The glycogen and triglyceride contents of larvae have been determined. Groups of 10 larvae were homogenized in 300 µL of homogenization buffer [0.01 M KH2PO4, 1 mM ethylenediaminetetraacetic acid (EDTA) pH 7.4] using a motorized Microfix mortar and a molten pipette tip as a pestle. The homogenates were centrifuged at 11 600 × g for 3 min. in order to pellet cellular and cuticular debris. Samples from the same homogenate were used for all assays.

Glycogen

Reagentia as available form the Sigma glucose-determining kit (PGO enzyme, catalog no. 510-6; Sigma-Aldrich Corp., St Louis, MO, USA) were utilized. The reagents were complemented by 0.1 U/mL amylloglucosidase, for converting glycogen into glucose. A 30 µL sample of the homogenate was added to a total volume of 1 mL test reagents. Following 30 min of incubation at 37 °C, the absorption at 450 nm was measured.
Triglycerides
Reagentia as available from the Sigma triglyceride determining kit (catalogue no. 336-20) were used for the colorimetric determination of the triglyceride content. A 30 µl sample of the homogenate was added to a total volume of 1 mL test reagents. Following 30 min of incubation at room temperature, the absorption at 500 nm was measured.

Statistical analysis
Probit regression analysis was performed on the pupation probabilities with larval weight or larval age as independent variables to compare critical weight and age (weight and age at which 50% of the larvae successfully pupate) between the geographical populations. These statistics are measures for the size and age of larvae necessary to reach the developmental stage that allows for pupation. The age of the larvae is expressed in stages (1 through 5) rather than hours to enable pooled analysis of data from the different rearing temperatures. Pupation probabilities were probit transformed prior to further analysis. Partial Pearson correlation coefficients were calculated between pupation frequencies and adult body size controlling for larval size and age and between pupation frequencies and larval size or age adjusting for the other variable. The former analysis allows for estimating a phenotypic trade-off between adult body size and pre-adult survival. The latter procedure allows for estimating the effect of larval size and of developmental stage on pupation probability (i.e. pre-adult survival), independently from each other. On the data aggregated over vials, a partial correlation test was used to detect a relationship between probit transformed pupation frequencies, adult fresh and dry weight and larval mass-specific triglyceride and glycogen levels. In vials with only very few larvae emerging, adult weight was higher than the average weight of the larvae placed in the vial, despite the absence of food. The few survivors were probably cannibalizing on the others. These vials were removed from the analysis.

Results

Larval weight, age and probability to pupate
Probit analysis of the data on larval weight, age and pupation probability did not reveal a significant difference in critical weight or age (weight or age at which 50% of the larvae pupate) between the populations (Table 1). The populations did differ, however, in the variable explaining most of the variation in pupation probability. Both size within age and age of the larvae influenced pupation probability (Fig. 1) but in a different manner for the tropical vs. the temperate populations. Calculating partial correlation coefficients allows for quantifying the contribution of each variable individually.

Table 1 Critical age and weight for pupation for the different geographical populations pooled over the corresponding developmental stages (timepoints) across rearing temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Critical age (stages)</th>
<th>95% CIV</th>
<th>Critical weight (mg)</th>
<th>95% CIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panama</td>
<td>1.42</td>
<td>0.42–2.11</td>
<td>0.28</td>
<td>−0.09–0.54</td>
</tr>
<tr>
<td>Congo</td>
<td>2.25</td>
<td>1.49–2.82</td>
<td>0.77</td>
<td>0.48–0.99</td>
</tr>
<tr>
<td>Denmark</td>
<td>1.48</td>
<td>0.49–2.11</td>
<td>0.47</td>
<td>0.11–0.72</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2.39</td>
<td>1.56–3.03</td>
<td>0.85</td>
<td>0.54–1.10</td>
</tr>
</tbody>
</table>

Fig. 1 Three dimensional regression planes for larval weight, larval age and pupation probability for the four geographical populations. Larval weight is presented on reverse axes.

For the tropical populations, the partial correlation of larval weight within age with pupation probability is appreciable and near significant (Congo: \( \rho = 0.473, \ P = 0.011 \), Panama: \( \rho = 0.375, \ P = 0.065 \)). In contrast temperate populations showed no correlation at all (Denmark: \( \rho = -0.013, \ P = 0.949 \), Netherlands: \( \rho = 0.06, \ P = 0.791 \)). Considering the partial correlation of larval age with pupation probability corrected for the effect of weight, the temperate populations showed moderately large and near significant correlation (Denmark: \( \rho = 0.456, \ P = 0.015 \), Netherlands: \( \rho = 0.412, \ P = 0.056 \)) opposite to the tropical populations (Congo: \( \rho = -0.157, \ P = 0.425 \), Panama: \( \rho = 0.136, \ P = 0.516 \)). The partial correlation coefficients of age and weight within age with pupation probability are summarized in Fig. 2 to quantify the alternative patterns of tropical vs. temperate populations also depicted in Fig. 1. Briefly, the tropical populations rely on converting accumulated
larval body mass to pupation probability against the temperate populations which do not enhance larval survival by utilizing accumulated resources.

**Pupation probability and prospective adult body size**

A negative partial correlation was found between pupation probability and adult fresh weight for all populations, controlling for larval size and age and calculating over rearing temperatures (Fig. 3). However, this correlation was strong and significant only for the temperate populations (Denmark: $\rho = -0.557$, $P = 0.003$, Netherlands: $\rho = -0.629$, $P = 0.002$). In Congo it was not significant ($\rho = -0.174$, $P = 0.385$) and in Panama the correlation coefficient was significant, but lower compared to the temperate populations ($\rho = -0.406$, $P = 0.048$). Considering the rearing temperatures separately reveals that this negative relationship is present predominantly at 17.5°C ($\rho = -0.632$, $P < 0.001$), resembling the pattern produced by the geographical populations. At 27.5°C the correlation is negative but nonsignificant ($\rho = -0.162$, $P = 0.197$).

These data again suggest a difference between the geographical populations in the details of determining not only pupation probability but adult body size as well. The temperate and tropical populations again exhibit an alternative pattern when considering the influence of pupation probability on adult weight at different stages of larval development as illustrated in Fig. 4.

**Larval glycogen content, pupation frequencies and prospective adult body size**

Larval mass-specific glycogen levels were partially correlated to both adult body size and pupation probability revealing an interesting pattern (Fig. 5). At the lower rearing temperature, the partial correlation between glycogen level and adult body size, controlled for age of the larvae (in hours) and calculated over the geographical populations, was strongly positive ($\rho = 0.713$, $P = 0.001$) but the partial correlation between glycogen level and pupation probability negative ($\rho = -0.575$, $P = 0.012$). At the high rearing temperature the pattern was reversed, with glycogen level negatively correlated with adult body size ($\rho = -0.514$, $P = 0.014$) and near significantly positively associated with pupation probability ($\rho = 0.339$, $P = 0.122$).

A similar, though less robust, pattern was found analysing the different geographical populations (Fig. 6). The tropical populations showed no significant partial correlation between glycogen level and either adult body size or pupation probability. However, the Netherlands population exhibits a strong positive partial correlation between glycogen level and adult body size ($\rho = 0.884$, $P = 0.012$).

![Fig. 2 Partial correlation coefficients for larval weight and pupation probability corrected for larval age and pupation probability corrected for larval weight.](image)
found in the Netherlands population and the correlation coefficients are sizeable.

In summary, the pattern of utilization of glycogen seems to be identical at 17.5 °C compared with the two temperate populations. Glycogen seems to be invested in adult body size at the expense of larval pupation probability. At 27.5 °C and in the two tropical populations no significant patterns are found.

**Discussion**

**Adult size and pupation probability: a life-history trade-off**

The aim of this study was to examine whether geographical variation in body size could be interpreted as a result of thermal evolution of resource allocation. A life-history trade-off mediated by adaptive resource allocation would provide an ultimate explanation. A physiological mechanism of allocation would provide a proximate explanation. A difference in resource allocation between geographic populations would be the starting observation.

The data showed a negative partial correlation between adult size and pupation probability when larvae of the same age and weight were considered. Partial correlations allow for the best possible comparison of different populations that might have different growth characteristics. The difficulty is that the same larval weight or age might have a different meaning for different genotypes, such that the same treatment might be experienced differently. Correcting for both age and weight seems the
proper way to circumvent this issue, but one needs to be aware of this complication. Bearing the previous in mind, higher probability of pupating was associated with lower adult size once feeding was stopped.

This pattern represents a life-history trade-off between pre-adult survival and adult size. Many life history trade-offs between pre-adult survival and adult size are known. In caddis flies (trichoptera), increased investment in larval defense has been shown to result in smaller adults (Stevens et al., 1999; Stevens et al., 2000). In these studies experimental manipulation of resource availability for pupation allowed the manifestation of a trade-off between pre-adult survival and adult size. Fellowes et al. (1999) found a similar trade-off. Drosophila melanogaster larvae that survived being parasitized by Asobara tabida emerged as smaller adults than unparasitized larvae. In both Drosophila and caddis flies, larval investment in defense decreased adult size. However, in our study no larval defense was involved. The present trade-off between larval survival and adult body size might be more comparable to a trade-off between early fecundity and adult longevity, under stress or without stress (Djawdan et al., 1996; Djawdan et al., 1998). This is a trade-off between a present fitness component and a reservation towards a future fitness component.

Determinants of pupation probability: geographical variation in resource allocation

Life-history trade-offs have often been thought to be the result of adaptive allocation of limiting resources (Atkinson & Sibly, 1997; Zera & Harshman, 2001). Our data support distinct thermal evolution of resource allocation. Populations from different geographical origins differed strikingly in the variable explaining most of the pupation probability. Larval weight explained most of pupation probability, but not adult size, in tropical populations. We interpret this as allocation of accumulated resources to survival rather than adult size. A minimal size is needed to pupate (Bakker, 1961) and this might vary between genotypes within populations, in our case within the tropical populations. A possible difference between populations would be that critical weight is lower in tropical populations than in temperate populations. An earlier study on variation in critical weight for pupation showed lower critical weight in a tropical population than in a temperate population (De Moed et al., 1999), but in our study no difference in critical weight was found (Table 1). In fact, within the temperate populations variation in larval weight did not explain variation in pupation probability, but rather correlated with adult weight. These larvae depended on age for their survival. The interpretation is that temperate populations preferentially allocate accumulated resources to adult size as opposed to larval survival. It seems unlikely that this result was because of lower availability of resources to larvae from temperate populations. Larval weights were not lower (data not shown) and previous results showed that temperate larvae accumulated larger lipid and triglyceride stores (Z. Bochdanovits and G. de Jong, unpublished data) (Verrelli & Eanes, 2001). A possible interpretation might be that tropical larvae are able to mobilize their resources more easily to facilitate pupation, compared with temperate larvae of similar age. If temperate larvae have been selected for producing large adults, they might fail to reallocate resources for survival once these resources are designated for future adult size. A similar energy reallocation hypothesis has been put forward to explain the trade-off between survival and reproductive investment in aphids (Stadler, 1995). When under food stress, young embryos might be reabsorbed by the mother, but once an embryo reaches a certain developmental stage it competes for resources, i.e. the mother is no longer capable of reallocating the energy previously designated for reproduction. In addition, adaptive variation in allocation among latitudinal populations has been shown before, in fish. Individuals from tropical populations of the Atlantic silversides (Menidia menidia) show submaximal growth rate but increased sustained and burst swimming capability, reducing predation risk by different allocation of resources (Billerbeck et al., 2000; Billerbeck et al., 2001; Lankford et al., 2001). In parallel with our findings, warm adapted genotypes M. menidia reduce investment in growth to increase survival.

Physiological basis of adaptive resource allocation

Evidence to support the argument of temperature dependent resource allocation might come from glycogen content in larvae at the weight and age they stop feeding. Larval mass-specific glycogen content correlated positively with adult size and negatively with pupation probability, at the lower rearing temperature. At the higher rearing temperature, the pattern was reversed. The correlation with glycogen content was interpreted as glycogen usage. If the interpretation of the correlations as glycogen usage is correct, glycogen usage might explain the trade-off between adult body size and survival. Glycogen would be the resource the two traits depend on. The data also provide some support for evolved differences of resource allocation patterns. Although the two tropical populations produce no significant correlation between glycogen level and adult size or pupation probability, the temperate populations approximate the expected pattern. In the Netherlands a significant and strong negative correlation between larval glycogen level and pupation probability was found, coinciding with a significant and strong positive correlation between larval glycogen level and adult size. The Denmark population produced the same trend. Larvae from temperate populations do not invest resources to propagate their own survival; instead they seem to invest in adult size. A similar metabolic basis of life history
variation has been documented in a wing-polymorphic cricket Gryllus firmus. Genetically determined higher lipid accumulation in flight capable morphs facilitates flight by providing fuel at cost of ovarian development compared with obligatory flightless morphs (Zera & Larsen, 2001).

The correlations with glycogen level are less conclusive for the geographical populations than for the rearing temperatures, but seem to support the hypothesis that differences in the ability to mobilize resources exist between populations. Lower temperature and temperate populations show a positive correlation between glycogen level in larvae and adult body size. The negative partial correlation between pupation probability and adult size was found predominantly at the low rearing temperature and for the temperate populations. The mechanisms of temperature related developmental plasticity in adult body size and temperature related geographic variation in body size might well be similar.

**Why larger size when cold?**

Cold adapted genotypes or cold reared larvae tended to maintain a higher prospective adult size at a cost of larval survival probability, and seemed to use stored glycogen preferentially to increase adult size. This suggests that a cold environment might select for larger adult size, whereas a warmer environment might select for higher larval survival. The trade-off seems understandable as large adult body size has been shown to confer higher fitness at low temperature only but not at high temperature. In a comparison of lines selected for body size, McCabe & Partridge (1997) found that the females from the large selection lines were relatively fitter at the colder temperature. At both experimental temperatures, especially the lower one, the small-line females rescheduled their progeny production to later ages. Reeve et al. (2000) found for the same lines that large-line males were fitter than controls at both temperatures. Interestingly, the difference in fitness was greater at the lower experimental temperature, showing genotype by environment interaction indicating a synergistic effect of larger body size and lower temperature. Larger body size may have evolved at temperate latitudes because of the fitness advantages of being larger at lower temperatures (McCabe & Partridge, 1997; Reeve et al., 2000). Nunney & Cheung (1997) compared early fecundity of female D. melanogaster. Test temperature for early fecundity was varied, as well as development temperature. Early fecundity was highest if the test temperature was equal to the development temperature, indicating that the developmental response to temperature was adaptive (Nunney & Cheung, 1997).

The reason why temperate populations accumulate larger glycogen stores per unit weight (Verrelli & Eanes, 2001; Z. Bochdanovits and G. de Jong, unpublished data) but seem to invest fewer resources in larval survival may be that temperate larvae are unable to access their reserves as quickly as tropical larvae. Adaptation to a warm climate may result in a larval physiology that allows for quick access of metabolic stores. The resulting pattern of resource allocation would be the one proposed, with temperate populations investing in size and tropical ones investing in survival.

This finding is in line with studies on clinal variation in functional differences in phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PD) allozymes. Higher PGM and lower G6PD activity is associated with higher latitudes and results in increased glycogen storage in adults (Eanes, 1999; Verrelli & Eanes, 2001). The same enzyme activities may be expected in larval metabolism as well, suggesting a possible metabolic mechanism for the observed physiological trade-off.

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