Relationship of larval density and heterozygosity to growth and survival of juvenile marbled salamanders (*Ambystoma opacum*)

Anne C. Chazal, John D. Krenz, and David E. Scott

Abstract: Intraspecific competition and enzyme variability have been observed to influence the bioenergetics of many organisms. In amphibians, larval growth affects body size at metamorphosis, which in turn can lead to differences in adult survival and fecundity. We manipulated larval density in a population of the marbled salamander, *Ambystoma opacum*, and measured body size and enzyme variability in surviving newly metamorphosed juveniles. Crowded larval conditions resulted in lower survival and smaller body sizes at metamorphosis. Multilocus heterozygosity showed no relation to body size at high larval densities; however, at low larval densities relatively homozygous animals were larger. There was a significant interaction between heterozygosity and larval density in their effects on larval traits. Competition had a greater effect on body size at metamorphosis than did heterozygosity. Survival may be enhanced by heterozygosity but in a manner unrelated to body size.

Résumé : La compétition intraspécifique et la variabilité enzymatique influencent la bioénergétique de plusieurs organismes. Chez les amphibiens, la croissance larvaire affecte la taille à la métamorphose, facteur qui pent à son tour entraîner des variations dans la survie des adultes et la fécondité. Nous avons modifié artificiellement la densité des larves au sein d'une population de Salamandres marbrées, *Ambystoma opacum*, et mesuré la taille du corps et la variabilité enzymatique chez les juvéniles qui ont servécu à la métamorphose. La densité élevée des larves donne lieu à des taux de survie plus faibles et à des tailles réduites à la métamorphose. L'hétérozygotie à plusieurs locus n'est pas reliée à la taille du corps dans des conditions de densité élevée mais, dans des conditions de densité faible, les animaux relativement homozygotes sont plus gros. L'hétérozygotie et la densité des larves ont une influence interactive significative sur les caractéristiques larvaires. La compétition a plus d'effet que l'hétérozygotie sur la taille à la métamorphose. La survie peut être accrue par l'hétérozygotie, mais sans qu'il y ait intervention de la taille. [Traduit par la Rédaction]

Introduction

Individual genetic variation has been correlated with fitness-related characteristics such as growth rate and survival (for reviews see Mitton and Grant 1984; Allendorf and Leary 1986; Zouros and Foltz 1987). Positive correlations between multilocus heterozygosity and fitness-related traits such as body size, growth rate, survival, and fecundity occur across a broad range of organisms, including plants (Mitton et al. 1981), bivalves (Koehn and Gaffney 1984; Koehn et al. 1988), fish (Danzmann et al. 1985), mammals (Garten 1977; Johns et al. 1977; Chesser and Smith 1987; Cothran et al. 1983, 1987; Scribner and Smith 1990), and amphibians (Pierce and Mitton 1982). The genetic and physiological processes underlying these whole-organism patterns are a matter of current controversy (for reviews see Mitton 1994; Zouros and Pogson 1994).

Multilocus heterozygosity (i.e., enzyme variability) may be related to metabolic efficiency, as suggested by significant negative correlations between heterozygosity and both oxygen consumption and protein biosynthesis rates (see Koehn and Shumway 1982; Hawkins et al. 1986, 1987; Koehn and Bayne 1989). High levels of heterozygosity might promote energy savings in the maintenance portion of the energy budget and lead to increased energy available for allocation to growth, reproduction, or storage.

Alternatively, other studies report either no association between multilocus heterozygosity and fitness characters or, in some cases, a homozygote superiority (Allendorf and Leary 1986; Booth et al. 1990; Rhodes 1991; Rhodes and Smith 1993; Gaffney 1990). Correlations between heterozygosity and fitness-related traits may not be temporally consistent and may be environment dependent. For example, Teska et al. (1990) found a relationship between heterozygosity and assimilation rates in old-field mice (*Peromyscus polionotus*) when fed a low-quality diet but no association was found when they were fed a high-quality diet. Associations between heterozygosity and fitness correlates might only be expressed in certain age groups or developmental stages (Allendorf and Leary 1986) or under particular environmental conditions (Allendorf and Leary 1986; Teska et al. 1990).

In amphibians, reported relationships between heterozygosity and fitness correlates are equivocal, and may also be dependent on age group, developmental stage, or environmental conditions. Pierce and Mitton (1982) found a positive relationship between heterozygosity and growth rate

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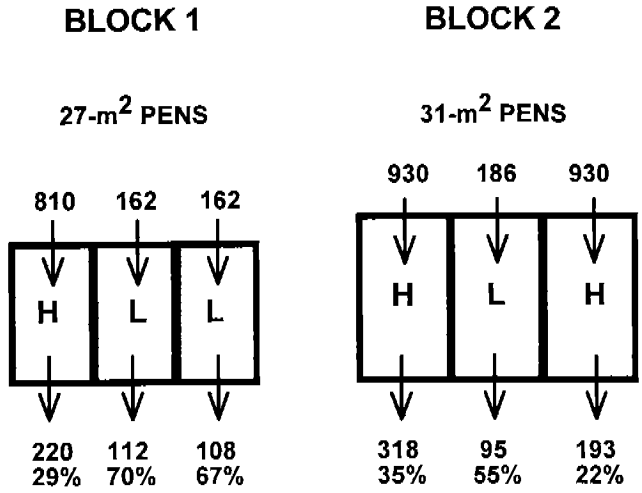
early, but not late, in the larval period of the tiger salamander, *Ambystoma tigrinum*. In another study of *A. tigrinum*, the more heterozygous animals consumed less oxygen at rest but consumed more oxygen during activity than did the less heterozygous individuals (Mitton et al. 1986). Samollow and Soulé (1983) describe consistent increases in population heterozygosity during the winter following metamorphosis in immature *Bufo boreas*, and these changes were mediated through differential mortality by genotype. However, no heterozygote advantage was evident for survival during either the larval or metamorphic period. Mitton and Grant (1984) proposed that an association between multilocus heterozygosity and individual performance is more likely to be detected under more stressful environmental conditions.

Laboratory and field experiments in amphibians have shown that larval density is an important environmental variable influencing amphibian development. Crowded (i.e., stressful) conditions result in a decreased larval growth rate, longer larval period, smaller body size at metamorphosis, and lower survival (Rose 1960; Brockelman 1969; Wilbur 1977; Travis 1980; Semlitsch and Caldwell 1982; Scott 1990). Associations between heterozygosity and larval traits may therefore be mediated by larval density.

Larval growth rate and body size are particularly important larval traits because of their association with survival to metamorphosis. More rapid growth and larger body size reduce the risk of predation (Caldwell et al. 1980), increase competitive ability to acquire limited resources (Wilbur and Collins 1973; Wilbur 1980), and allow earlier metamorphosis (Wilbur and Collins 1973; Wilbur 1977). Larval body size may affect adult performance in some species, as large metamorphs may have a higher rate of survival to sexual maturity and may mature earlier and at a larger adult size than small metamorphs. Larger females produce larger clutches and larger males have greater mating success (Howard 1978; Berven 1981; Berven and Gill 1983; Smith 1987; Semlitsch et al. 1988; Scott 1994). Thus, an association between heterozygosity and larval body size may have important consequences for individual fitness components.

In the marbled salamander, *Ambystoma opacum*, field experiments have demonstrated that competitive interactions at high aquatic densities result in slowed larval growth, smaller body size at metamorphosis, a longer larval period, and lowered larval survival (Scott 1990). Density effects persist in adults; individuals from a high-density larval treatment were smaller, matured later, and exhibited reduced adult survival (Scott 1994). Although the evidence suggests a strong influence of the larval environment on traits related to fitness (i.e., body size at metamorphosis and maturity, fecundity, and survival) for *A. opacum*, the importance of individual differences in levels of genetic variation is unknown. Our study examined larval traits of *A. opacum* known to be related to individual fitness and their relationship with multilocus heterozygosity under two environmental regimes (high and low larval density). These density levels are intended to represent two levels of competitive stress, and thus two biotic environmental treatments. We tested three hypotheses: (1) individual body size will be associated with individual multilocus heterozygosity (H_I), (2) individual body size will be influenced by an interaction of H_I and density, and (3) mean population heterozygosity (H_P) of survivors will differ between low- and high-density treatments, owing to differential survival among heterozygosity classes.

Fig. 1. Numbers of hatchling *Ambystoma opacum* stocked, and number and percentage surviving to metamorphosis in each field pen. Field pens were arranged in two blocks based on pen size and location in Ginger's Bay (for construction design see Scott 1990). Each pen was randomly assigned to either a low-density (L; 6 larvae/m^2) or a high-density (H; 30 larvae/m^2) treatment, with the restriction that each block contain both treatments.



Methods

Density manipulations

Our study was conducted at Ginger's Bay, a 0.8-ha Carolina bay (Sharitz and Gibbons 1982) located on the Department of Energy's Savannah River Site in Aiken County, South Carolina. Ginger's Bay often dries in spring or summer and refills in late autumn or early winter (Scott 1993). During this study, Ginger's Bay began filling in mid-October 1990 and remained full throughout the summer of 1991. Therefore, *A. opacum*, which typically have a larval period of 4–6 months (Scott 1990), had an adequate hydroperiod to develop and metamorphose successfully.

On 10 October 1990, 90 adult females and 60 adult males entered Ginger's Bay to breed and were captured in pitfall traps along a drift fence that encircled the breeding site (Gibbons and Semlitsch 1981). Females and males were evenly and randomly apportioned into three 2 m diameter galvanized steel tanks containing soil and vegetative cover. Beginning on 14 December (and again on 24 and 31 December) 1990, ≈2500 eggs were removed from each tank, mixed with eggs from other tanks, and submerged in plastic pools (1.5 m diameter). Most eggs hatched within 48 h.

On 18 December 1990, 27 December 1990, and 2 January 1991, hatchlings were drawn randomly from the pools and distributed among six field pens (Fig. 1). At the same time, we randomly collected and froze ≈100 individuals from the stock population to provide an initial estimate of heterozygosity. However, owing to a freezer malfunction and consequent tissue degradation, only samples collected on 2 January 1991 and placed in a separate freezer (n = 47) remained intact.

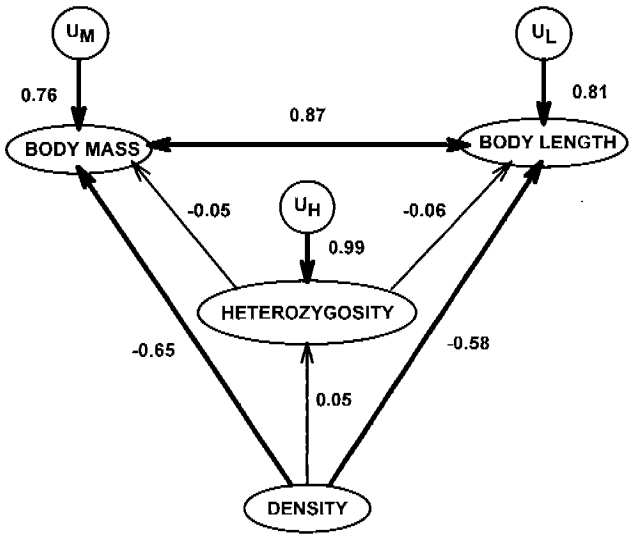
Metamorphosed *A. opacum* (n = 1046) were captured between 12 April and 17 May 1991 in minnow traps placed along the pen walls with the funnel openings at the water surface. We recorded snout–vent length (SVL; ±0.5 mm) and body mass (±0.01 g) and removed 0.7 cm of tissue from the tail tip for genetic analysis. The tissue sample was frozen at −60°C prior to electrophoresis.

Table 1. Mean of body mass and length, survival, and larval period for metamorphosed *Ambystoma opacum* under two treatments.

Treatment	Mass (g)	SVL (mm)	Survival	Larval period (days)
Low density ($n = 3$)	2.53 (0.10)	44.4 (0.40)	0.64 (0.04)	112.9 (1.9)
High density ($n = 3$)	1.65 (0.10)	39.7 (0.50)	0.28 (0.04)	116.2 (2.3)
$F_{[1,4]}$	33.6*	37.2*	114.7**	0.8 ns

Note: F values and significance levels are from ANOVA of treatment means. Numbers in parentheses show the standard error. *, $P < 0.01$; **, $P < 0.001$; ns, not significant.

Fig. 2. Diagram of path analysis (Kingsolver and Schemske 1991) used to evaluate the relative contributions of individual heterozygosity and larval population density to body mass and body length of metamorphic *Ambystoma opacum*. A single-headed arrow indicates a causal path and a double-headed arrow a correlation path. The line width indicates the magnitude of the effect. All manifest variables (ovals) are measured, therefore the path coefficients are standardized partial-regression coefficients. Exogenous latent variables (circles) include all unmeasured variables that affect a measured variable and represent error variances (U_M, U_L, and U_H symbolize all unmeasured variables that affect body mass, body length, and heterozygosity, respectively). Path coefficients for exogenous latent variables were calculated as $\sqrt{(1-R^2)}$, where R is the proportion of variation in the dependent variable that is explained by the model.



Because hatchlings were introduced to the pens over a 2-week period to simulate gradual pond filling, the mean larval period for each pen was calculated as the number of days from the average date of introduction of hatchlings to the mean date of metamorphosis. We did not test for a relationship between H_I and larval period, as Whitehurst and Pierce (1991) did, because the larval period for a particular individual could not be determined without a relatively large error.

Genetic analysis

Allozyme genotypes were determined for each hatchling and metamorphosed juvenile using horizontal starch gel electrophoresis (Selander et al. 1971). Twenty-four allozyme loci were resolved consistently. Nine loci were polymorphic and used to estimate multilocus heterozygosity: aspartate aminotransferase (*Aat*), glucose-6-phosphate dehydrogenase (*Gd*), glucose phosphate isomerase (*Gpi*), isocitrate dehydrogenase (*Idh*), lactate dehydrogenase (*Ldh*), malate dehydrogenase-1 and -2 (*Mdh-1* and *Mdh-2*), malic enzyme (*Me*), and peptidase (*Pep*). Electrophoretic techniques followed those described by Manlove et al. (1975; *Aat*, *Gd*, *Gpi*, *Ldh*, *Mdh-1*, and *Mdh-2*), Selander et al. (1971; *Idh* and *Me*), and Harris and Hopkinson (1976; *Pep*). We determined the genotype of 849 salamander metamorphs from the density manipulations. Mean heterozygosity per locus was 0.234 ± 0.073, and ranged from 0.010 (*Idh*) to 0.610 (*Pep*); the mean at five loci was >0.250. The allozymes detected were shown to be heritable by the analysis of independent mother–offspring sets (J.D. Krenz, unpublished data).

Statistical analysis

To examine the relationship between levels of individual heterozygosity (H_I) and body-size traits, we specified linear and curvilinear (i.e., quadratic) contrasts in a two-way analysis of variance (ANOVA) model that included density level, heterozygosity class, and their interaction. In several analyses, a significant density × H_I interaction was observed; consequently, H_I effects were assessed separately for each density level. Linear and quadratic contrasts were specified to maintain ordering in H_I classes, because relationships (if present) were expected to be linear or curvilinear.

Mean population heterozygosity (H_P) was calculated as the mean of H_I/24 (the number of loci we assayed) for each population. We used ANOVA to examine the effect of density on mean population heterozygosity (H_P), larval period, body size (SVL and mass) at metamorphosis, and larval survival (the number of metamorphs in each pen population divided by the number of hatchlings introduced). Survival data were arcsine square-root transformed for statistical analysis. We used the pen × density interaction as the error term to test for the main effect of density; this procedure is tantamount to using pen means to test for density effects (Wilbur 1987; Scott 1990). All ANOVA tests were performed using the general linear models procedure of the Statistical Analysis System (SAS Institute Inc. 1985).

We used path analysis (Kingsolver and Schemske 1991) to assess the relative effect of heterozygosity and larval density on body-size traits. For the dependent (body mass and SVL) and independent (H_I and density) measured variables, path coefficients were standardized partial-regression coefficients obtained from multiple regression analysis (Steiger 1989). Our a priori path diagram is presented in Fig. 2.

For each pen and treatment, χ^2 tests of significance of deviations of observed genotypic frequencies from Hardy–Weinburg equilibrium expectations were calculated using the Biosys-1 computer program (Swofford and Selander 1981).

Results

Observed genotypic frequencies were not significantly different from Hardy–Weinburg equilibrium expectations for any loci for either treatment. Mean heterozygosity per locus

Fig. 3. Mean body length (SVL) of metamorphosed *Ambystoma opacum* for each heterozygosity class by density treatment. Vertical bars indicate 1 SE.

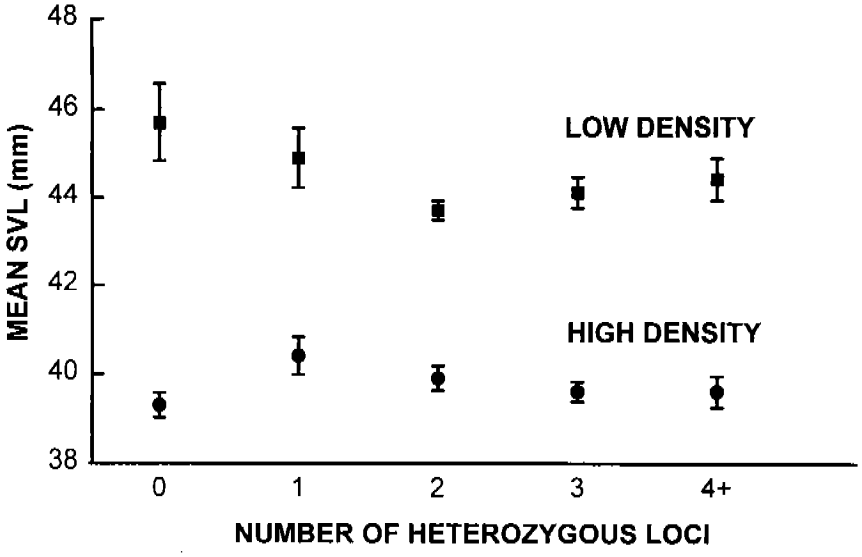


Table 2. ANOVA of body size of *Ambystoma opacum* reared at two densities and genotyped at metamorphosis, and linear and quadratic contrasts for the interaction between H_I and density.

Source of variation	df	Response variable	Sum of squares	F	P
H_I	5	Mass	2.36	2.38	0.037
		SVL	99.92	2.50	0.029
H_I × density	5	Mass	2.47	2.50	0.029
		SVL	90.07	2.26	0.047
Error	833	Mass	164.81		
		SVL	6653.35		
H_I × density contrasts					
Linear	1	Mass	0.00	0.01	0.921
		SVL	1.04	0.13	0.718
Quadratic	2	Mass	2.02	5.10	0.006
		SVL	61.12	3.83	0.022

Table 3. Low- and high-density treatment contrasts (linear and quadratic) of body mass and length (SVL) for individual heterozygosity in metamorphosed *Ambystoma opacum*.

Source of variation	df	Response variable	Sum of squares	F	P
Low density					
Linear	1	Mass	0.07	0.16	0.688
		SVL	0.00	0.0	0.999
Quadratic	2	Mass	3.05	3.35	0.036
		SVL	86.61	3.13	0.045
Error	281	Mass	127.59		
		SVL	3889.20		
High density					
Linear	1	Mass	0.01	0.14	0.710
		SVL	0.78	0.14	0.710
Quadratic	2	Mass	0.21	1.12	0.328
		SVL	4.25	0.39	0.678
Error	556	Mass	51.07		
		SVL	3038.76		

was 0.234 ± 0.073, and ranged from 0.010 (*Idh*) to 0.610 (*Pep*); mean heterozygosity at five loci was >0.250.

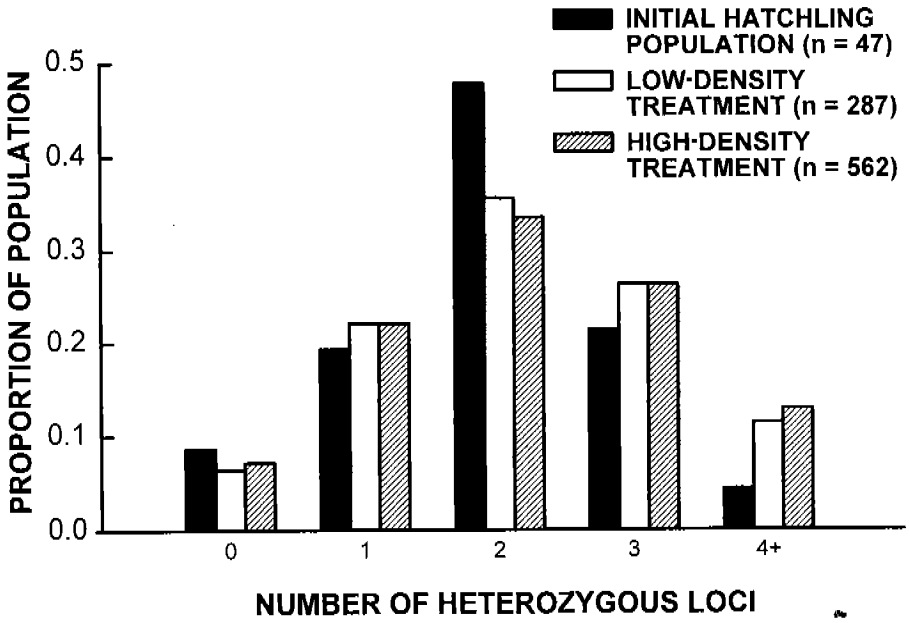
Larval density significantly affected larval body size at metamorphosis and survival (Table 1). Newly metamorphosed animals from the low-density treatment were heavier and larger than those from the high-density treatment. In addition, survival was greater in the low-density pens than in the high-density pens. We did not observe any effect of density on larval period.

In a reduced one-way ANOVA model with only H_I as a class variable, there was no effect of H_I on larval body size (mass: $F_{[5,843]} = 1.34$, $P = 0.24$; SVL: $F_{[5,843]} = 1.85$, $P = 0.10$). However, in a full model that included density and the density × H_I interaction term, the relationship between H_I and body size (both mass and SVL) was significant (Table 2), which indicated that the effect of H_I on mass and SVL depended on density level. At high density, neither the linear nor the quadratic contrast was significant (Table 3). At low density, individuals with intermediate and high heterozygosity levels were smaller; the largest animals tended to be the least heterozygous (Fig. 3).

Although there was an indication of higher mean H_P in the high-density pen populations, the difference between low and high densities was not statistically significant (H_P = 0.085 ± 0.003 and 0.090 ± 0.002 for low and high density, respectively; $F_{[1,4]} = 2.81$, $P = 0.17$; Fig. 4). The mean H_P level of the metamorphs was not significantly different ($F_{[2,893]} = 1.46$, $P = 0.23$) from the initial H_P of the hatchlings (0.081 ± 0.006; $n = 47$; Fig. 4).

Path analysis indicated strong negative relationships between larval density and body size, with weaker relationships between individual multilocus heterozygosity and body size. The standardized partial-regression coefficients between density and SVL and body mass were −0.58 and −0.65, respectively; the coefficients between H_I and SVL and body

Fig. 4. Proportion of populations in each heterozygosity class. Populations are the initial hatchling pool and cohorts surviving to metamorphosis in the low-density and high-density treatments. Mean population heterozygosity (H_P) was calculated as the mean of $H_I/24$ (the number of loci we assayed) for each population. Mean H_P is 0.081 ± 0.006 for the hatchlings, 0.085 ± 0.003 for low-density treatment, and 0.090 ± 0.002 for high-density treatment.



mass were −0.06 and −0.05, respectively (Fig. 2). Thus, larval density explained about 33 and 42% of the variance in SVL and body mass, respectively, whereas H_I explained <1% of the variation in these body-size measures.

Discussion

Individual heterozygosity

Observations of an interaction between heterozygosity and environment are not uncommon. A positive correlation between fitness-related traits and greater heterozygosity has been found in many studies and is usually more pronounced in more stressful environments. Stress is considered to be an environmental condition that causes less energy to be devoted to growth or reproduction than to maintenance (Koehn and Bayne 1989), and differences among individuals in energy acquisition and allocation patterns may be related to levels of heterozygosity. Energy differences have been demonstrated in taxa such as oysters (Koehn and Shumway 1982; Rodhouse and Gaffney 1984), mussels (Diehl et al. 1986), and old-field mice (Teska et al. 1990).

In larval salamanders, acquired energy is allotted to the competing compartments of maintenance (i.e., standard metabolism and activity metabolism, the latter including the costs of acquisition, digestion, and absorption of food) and production (i.e., a combination of increases in somatic tissue and, potentially, energy stores in the form of nonstructural lipids). Our study reports a significant interaction between H_I and larval density in their effects on larval body size; we observed a significant curvilinear relationship between body size and H_I only in the less stressful environment, with more homozygous individuals attaining a larger size. Although introducing the hatchlings to the enclosures over a 2-week period may have slightly increased the variance in size at metamorphosis in all heterozygosity classes, we obtained no evidence that the methodology obscured a potential relationship between H_I and size at metamorphosis.

The larger size of homozygous (compared with heterozygous) metamorphs surviving low larval densities may be due to any of several mechanisms. Homozygous individuals may have a longer larval period, may be better at acquiring resources, may have higher assimilation efficiencies, or may incur a lower metabolic cost due to activity. Any of these would allow homozygous animals to devote a greater amount or a higher proportion of their absolute energy intake to growth.

The stress that we imposed was tantamount to changing resource availability; relatively abundant per-capita food (zooplankton) was available at low salamander density, whereas fewer zooplankton were available at high salamander density and they were more mobile species (Scott 1990). It is unlikely that resource acquisition ability or assimilation efficiency were altered by our treatments, i.e., if homozygous individuals were better at catching or assimilating prey at low density, then they should enjoy a similar advantage at high density. It is more likely that the explanation for our observed pattern hinges on the relationship between activity metabolism and heterozygosity level. Although Mitton et al. (1986) reported that more heterozygous metamorphic *A. tigrinum* consumed oxygen more slowly when they were quiescent and food was unavailable than did homozygous individuals, the reverse was observed during activity. The latter is likely more representative of field conditions. In our study, if equal amounts of food are available to all individuals at low density (regardless of H_I) in a resource-rich environment, then a trade-off between growth and the costs of active metabolism could result in larger body size for homozygous individuals.

Although starvation studies have indicated that the efficiency of standard metabolism may be positively correlated with heterozygosity, other results (i.e., Mitton et al. 1986; Rhodes 1991; this study) suggest that under active conditions, relatively homozygous individuals may be more energetically efficient. Efficiencies when food is available or during activity may mask energy patterns due to starvation because the cost of standard metabolism is lower than the cost of activity metabolism. In the mussel *Mytilus californianus*, 25% of oxygen consumption was associated with standard metabolism, while 75% was due to the costs of feeding (water filtration, food digestion and absorption; Bayne and Newell 1983). Standard costs were met by 8% of the ingested ration, but feeding costs consumed 48%. In the terrestrial salamander *Plethodon jordani*, oxygen consumption rates increased 77% following a single feeding, were sustained for 6 days, and had not returned to fasting levels after 11 days (Feder et al. 1984). Additionally, the cost of locomotion can be greater than that of standard metabolism; active adult *Ambystoma* spp. expend 2- to 10-fold the energy spent at rest (see review by Gatten et al. 1992).

At high density, where zooplankton levels decrease and the prey community is dominated by highly mobile zooplankton species, the more heterozygous individuals may acquire more food than their homozygous counterparts, if higher activity metabolism is associated with greater mobility or faster movement. In this case their increased metabolic cost would be counterbalanced by higher food intake. Partial support for greater food consumption ability in heterozygotes comes from studies of the physiological energetics of marine mollusks. Generally, although the relatively heterozygous animals outgrow homozygous individuals, they achieve this not just through lowered absolute maintenance costs, but through higher efficiencies of food absorption and protein synthesis coupled with elevated water-filtration rates and consequently greater food consumption (Garton 1984; Garton et al. 1984; Hawkins et al. 1986). These studies suggest, as does that of Mitton et al. (1986) for *A. tigrinum*, that heterozygotes have greater "scope for activity" (i.e., the amount of energy available for processes other than standard metabolism). In our study, it is plausible that a greater scope for activity of heterozygotes may be more important under high-density conditions. An association in salamanders between heterozygosity, activity metabolism, and a physiological correlate such as sprint speed might also explain differential survival among heterozygosity classes at high density, if it occurs. Of course, this entire scenario is speculative, and an explanation of the patterns we have observed requires tests of the relationship between H_I, metabolic rate, and physiological performance measures.

Population heterozygosity

The effect of larval density on body size and survival provides evidence that the high-density environment was stressful compared with the low-density environment. At high density, survivors exhibited slightly higher H_P levels, although the difference between high- and low-density treatments was nonsignificant. However, the power of our ANOVA (with only three replicates) was low. We evaluated the power of our analysis using observed values of H_P for each treatment and the common standard deviation of the populations. For an effect size of approximately 0.6–0.8 (see Cohen 1977), the power of our analysis was $<30\%$. Nine replicates would be required to increase the power to $\approx 80\%$. Our results are therefore ambiguous because our a priori probability of rejecting the null hypothesis was small. The possibility of a weak relationship between H_I and density remains.

Genetic versus environmental determinants of body size

What relative contributions do density (competition) and heterozygosity (genetic) effects make to individual body size at metamorphosis? Welden and Slauson (1986) distinguish between the intensity and the importance of competition as "the amount of strain competition induces in an organism" versus "the relative degree to which competition contributes to the overall decrease in growth rate, metabolism, fecundity, survival or fitness of that organism below its optimal condition." They also note that some of the decrease in a trait such as growth rate is due to factors other than competition (e.g., genetic, environmental heterogeneity, predation, and chance).

An indirect measure of the importance of a particular process to the trait under examination is the proportion of variance explained by that process (Welden and Slauson 1986). Using our experimental design and path analysis, we calculated the proportion of body-size variation explained by density and multilocus heterozygosity. In this study larval density explained 42% of the variation in body mass and 34% of SVL. In contrast, H_I explained $<1\%$ of the variation in each of these traits. In studies where heterozygosity is found to be significantly correlated with individual traits such as body size at metamorphosis, H_I typically explains only about 5–10% of the variation (Garton et al. 1984; Koehn et al. 1988).

It is evident that under these experimental conditions (and specifically at this temporal scale), competition effects a greater strain on larval body traits than does the level of enzyme variability. Further, the relative role of competition is likely to be underestimated because competition probably occurs at larval densities below that of our low-density treatment, and because competitive effects have explained $\approx 80\%$ of the variance in body-size traits in other years at Ginger's Bay (Scott 1990).

The question of whether differential mortality occurs among heterozygosity classes is critical to the interpretation of our results. Previous studies have demonstrated strong effects of density on larval survival that appear to be mediated through effects on body size (Brockelman 1969; Wilbur 1977; Semlitsch and Caldwell 1982; Scott 1990). Scott (1994) has demonstrated for *A. opacum* from Ginger's Bay that differences in body size due to larval competition continue to affect an individual as an adult. Animals reared at high larval densities are smaller and exhibit delayed reproductive maturity and reduced survival. If there is no relationship between H_I and the probability of survival, it is clear that larval density is of paramount importance through its influence on body size, and individual heterozygosity is relatively unimportant in a heterogeneous environment.

However, if the homozygous individuals that were large at low density did not survive at high density and we were unable to detect this because of low statistical power, then heterozygosity may be very important to survival. Larval *A. opacum* with greater heterozygosity may be more likely

to survive the larval period, but in a manner unrelated to body size. Under stressful conditions we observed no relationship between individual heterozygosity and body size; however, an influence of heterozygosity on survival may be mediated not through enhanced growth but through altered energy allocation.

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