QUANTATIVE GENETICS OF GROWTH AND DEVELOPMENT IN THE FRESHWATER COPEPOD DIAPTOMUS LEPTOPUS

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Saran Twombly
Nancy Tisch

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Abstract:
Growth and development determine age and size at important life-history transitions and therefore contribute directly to individual fitness. Opportunities for these traits to respond to selection depend on the amounts of genetic variation present and on the extent to which they are regulated by the same genes, or correlated. In previous studies of the freshwater copepod *Diaptomus leptopus*, we found that age at metamorphosis and maturity - but not body size at these transitions - contributed to individual fitness, and we predicted that development - but not growth - should be sensitive to selection in field populations. Here we report estimates of broad sense heritabilites and genetic correlations for traits associated with growth and development obtained from laboratory, full sib matings. We detected significant genetic variation for traits related to development but not for those related to growth. Few genetic correlations were significant. Notably, growth and development were negatively correlated during the larval phase, but not among juveniles. Larval and juvenile growth rates showed no significant correlations, and neither age nor size at metamorphosis was correlated significantly with age or size at maturity. Our data show that ample variation exists for natural selection to modify development rates or ages at important transitions, and that few constraints prevent independent evolution of size and age, particularly at maturity. They also provide the first evidence that complex life cycles - and metamorphosis in particular - break genetic correlations between traits, allowing traits to respond independently to selection over ontogeny.

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Quantitative genetics of growth and development in the freshwater copepod *Diaptomus leptopus*

Saran Twombly

Department of Biological Sciences

University of Rhode Island

Kingston, Rhode Island 02881

(401) 874-2609; twombly@uri.edu

and

Nancy Tisch

Biometrics Department

Cornell University

Ithaca, New York 14853-7801

(607) 255-7826; nt34@cornell.edu

Right running head: Quantitative genetics of growth and development

Left running head: Twombly and Tisch

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Abstract

Growth and development determine age and size at important life-history transitions and therefore contribute directly to individual fitness. Opportunities for these traits to respond to selection depend on the amounts of genetic variation present and on the extent to which they are regulated by the same genes, or correlated. In previous studies of the freshwater copepod *Diaptomus leptopus*, we found that age at metamorphosis and maturity – but not body size at these transitions – contributed to individual fitness, and we predicted that development – but not growth – should be sensitive to selection in field populations. Here, we report estimates of broad sense heritabilities and genetic correlations for traits associated with growth and development obtained from laboratory, full sib matings. We detected significant genetic variation for traits related to development but not for those related to growth. Few genetic correlations were significant. Notably, growth and development were negatively correlated during the larval phase, but not among juveniles. Larval and juvenile growth rates showed no significant correlation, and neither age nor size at metamorphosis was correlated significantly with age or size at maturity. Our data show that ample variation exists for natural selection to modify developmental rates or ages at important transitions, and that few constraints prevent independent evolution of size and age, particularly at maturity. They also provide the first evidence that complex life cycles – and metamorphosis in particular – break genetic correlations between traits, allowing traits to respond independently to selection over ontogeny.

Introduction

The processes of growth and development determine the size and the age at which major life cycle transitions (e.g., metamorphosis, maturity) are accomplished, and therefore are important components of an organism's life-history. The fitness contributions of age or size at metamorphosis (e.g., Blakley 1981; Berven and Gill 1983; Smith 1987; Bradshaw and Holzapfel 1992; Taylor et al. 1998), size (e.g., Peters 1983; Mitchell-Olds 1986) and age (e.g., Cole 1954; Roff 1992) at maturity are often substantial. Despite these documented contributions to fitness, age and size are variable in many populations (e.g., Wilbur and Collins 1973, Chambers and Leggett 1987; Forrest 1987; Reznick 1990; Newman 1992; Twombly 1995). This variation is, at first, counterintuitive; fitness traits are assumed to be molded by selection so that individual values cluster tightly around population mean values and individual variation is low (Fisher 1958).

Phenotypic variation in growth and development can have both genetic and environmental bases, either of which can account for differences in size or age at important transitions. Environmentally–induced variation forms the basis for phenotypic plasticity (short-term, non-genetic phenotypic variation), whereas genetically-based variation provides the raw material for natural selection. The potential for any trait to respond to selection depends on the amount of genetic variation present for that trait and on the degree of correlation or covariance between traits (e.g., Lande and Arnold 1983; Palmer and Dingle 1986; Simons and Roff 1994).

The genetic basis for life-history traits, many of which are polygenic, is usually measured as heritability, h^2, the proportion of phenotypic variation that is due to genetic effects (Falconer

1981; Roff 1997). The expectation, following Fisher's Fundamental Theorem, is that heritability for life-history traits should be low (e.g., Falconer 1981; Hegmann and Dingle 1982; Mousseau and Roff 1987). This notion was verified in a survey of h^2 estimates for 75 species: life-history traits exhibited lower additive genetic variation than morphological or behavioral traits (Mousseau and Roff 1987). These authors also found a close correspondence between broad- and narrow-sense heritability estimates for different types of traits, suggesting that either estimate indicates evolutionary potential. The assumed pattern of lower heritability for life-history traits is not universal, however; in some organisms or some environments, h^2 estimates for life-history traits are high (e.g., Primack and Antonovics 1981; references in Berven 1987; Blouin 1992), and adequate genetic variation for selection to act on life-history (fitness) traits often exists (e.g., Palmer and Dingle 1986; Mousseau and Roff 1987; Travis et al. 1987; Tucic et al. 1988; Snyder 1991; Schwaegerle and Levin 1991; Simons and Roff 1994).

Price and Schluter (1991) offered an alternative explanation for low heritabilities of fitness traits, many of which (e.g., survival, fecundity) are the consequence of more than one metric trait. In *Geospiza fortis*, for example, survival is affected by body size (which is heritable) and by a number of environmental factors. Increased environmental variance, rather than decreased genetic variance, accounts for low heritability estimates in this species (Price and Schluter 1991; Simons and Roff 1994). In this case, the potential for body size to respond to selection exists even though heritability estimates are small. These authors conclude that there is no simple prediction about the magnitude of heritability estimates expected for life-history or fitness traits – a conclusion that fits with the range of estimates reported in the literature.

The interdependence of two traits (such as growth and development) is often inferred from phenotypic correlations. For example, significant negative correlations between age and size at metamorphosis have been interpreted as evidence that the processes of growth and development are coupled (e.g., Collins 1979; Travis 1984; Beck 1997). Because growth and development are plastic, however, and because correlations between these processes or their proxy measures (size and age) change with environmental conditions, phenotypic correlations may provide a poor indication of underlying genetic relationships (Reznick 1985; Stearns and Koella 1986). The extent to which two traits covary – that is, are determined by the same genes and evolve in concert– is best determined by estimation of genetic correlations or covariances (Falconer 1981, Roff 1996), or by direct mapping of the quantitative trait loci involved (Mitchell-Olds 1996).

For life-history traits, which often are under strong selection in the same direction, genetic correlations are expected to be negative more frequently than are correlations for other types of traits (Roff 1996). Although this prediction is supported by some empirical evidence (Roff 1996), several studies have found positive genetic correlations between fitness traits such as growth and development (e.g., Blouin 1992; Dorn and Mitchell-Olds 1992). These correlations vary between populations (Berven 1987) as well as between phases of an individual's life cycle (Roach 1986). Interpreting genetic correlations can be difficult, however. The estimates are often imprecise (e.g., Palmer and Dingle 1986; Simons and Roff 1996), may be exaggerated by exposure to novel environments (e.g., Service and Rose 1985; Holloway et al. 1990), and may not represent the underlying genetic architecture or co-evolution between traits (Houle 1991).

For organisms with complex life cycles, estimates of heritability and genetic correlations between traits expressed at different developmental stages assume an additional importance because they indicate the extent to which different phases of an organism's life cycle can respond independently to selection (e.g., Ebenman 1992). Selection often acts in different directions or intensities during different portions of an organism's life cycle. For example, Price and Grant (1984) found that natural selection favored small body size in juvenile *Geospiza fortis* but large body size in adults, and Roach (1986) found consistent, negative genetic correlations between traits expressed in juvenile *versus* adult stages in *Geranium carolinanum.* In addition, a suite of traits expressed by early juvenile stages correlated negatively with fecundity whereas the same traits, expressed in adults, correlated positively with fecundity. Negative genetic correlations between a trait expressed during two different life cycle phases may constrain or counteract the effects of selection on either trait (Lande and Arnold 1983). Ebenman (1992) used an ESS model to propose that complex life cycles, and metamorphosis in particular, evolved to break these genetic correlations, allowing selection to act independently on larval and juvenile or adult phases. In the green tree frog, *Hyla cinerea* , larval and juvenile growth rates were not genetically correlated and Blouin (1992) suggested that juvenile growth rate was free to evolve independently. These studies, along with several showing that diverse larval morphotypes metamorphose into similar adult morphs, or vice versa (see references in Ebenman 1992), support the ideas that 1) the direction of selection varies with trait and ontogenetic stage and 2) traits expressed in different life cycle stages of organisms with complex life cycles are free to respond independently to selection. For the most part, Ebenman's (1992) proposal remains untested.

In this study, we estimated heritabilities and genetic correlations for traits associated with growth and development for a freshwater crustacean, *Diaptomus leptopus*. Many crustaceans, including copepods, have complex life cycles that include metamorphosis. Larval duration is generally short and most growth occurs after metamorphosis. In recent laboratory studies, we have found that body size – either at metamorphosis or maturity – contributes little to individual (female) fitness (Twombly and Tisch, submitted manuscript). These results are in marked contrast to those from many other organisms (e.g., Peters 1983; Dorn and Mitchell-Olds 1992; Bradshaw and Holzapfel 1992; Taylor et al. 1998), but agree with the relative unimportance of size at metamorphosis or maturity in insects that continue to feed after pupation (e.g., McPeek 1990; Anholt 1991; Anholt et al. 1991; McPeek and Peckarsky 1998). Age at maturity contributed to lifetime female fitness. Age at metamorphosis was significantly correlated with age at maturity, suggesting that it also affects fitness. Based on these fitness contributions, we predicted that development – but not growth – should be the target of natural selection in field populations of *D. leptopus*. Individuals should metamorphose and mature early, irrespective of body size (Twombly and Tisch, submitted).

The potential for selection to modify developmental trajectories depends on the amount of (additive) genetic variance present for this trait and the extent to which this trait is correlated with others that may also be under selection. In the experiments described here, we raised full-sib families in two different food conditions and estimated broad-sense heritabilities for a number of traits associated with growth (larval and juvenile growth rates, size at metamorphosis and size at maturity) and development (age at metamorphosis and maturity). Based on previous

estimates of h^2 for fitness traits (Mousseau and Roff 1987), we expected to find low heritability for development or its proxy measures and higher h^2 values for body size. We also estimated genetic correlations among traits to determine the extent to which growth and development are coupled in *D. leptopus*, as well as the potential for selection to act independently on either age or size during different phases of the copepod life cycle. We again expected to find, based on previous measures (Roff 1996), negative genetic correlations between important life-history traits. Finally, we predicted that genetic correlations between age and size at metamorphosis and at maturity should differ, and that correlations between age or size at different ontogenetic stages should be low (Ebenman 1992).

Materials and Methods

Parental and Offspring (F1) Generations

Ovigerous female *Diaptomus leptopus* were collected from Little Bullhead Pond, Perryville, RI, in June 1999 and isolated in 20 mL modified MBL medium. Once eggs hatched, larvae (nauplii) were raised individually in 10 mL modified MBL medium, in small petrie dishes, in two different food conditions: low food (0.2 μg C/ml) and high food (0.6 μg C/ml). Both temperature (19°C± 2°) and photoperiod (14 Hr L:10 hr D) were held constant in our incubators. Individuals were observed daily, and food and medium were changed every second day. We quantified size at metamorphosis for each individual as the total length of the exuvium shed at the last larval (naupliar or N6) stage (Twombly and Burns 1996) and age at metamorphosis as the days after hatching that this molt occurred. Size and age at all subsequent juvenile molts

were similarly recorded for each individual; size and age at the ultimate molt (copepodite stage 5 or C5) were recorded as size and age at maturity.

Following the ultimate molt, each individual was paired with a non-sibling copepod raised on the same diet (1 female paired with 1 male, or full-sib mating design), and pairs were maintained in 30 mL modified MBL medium at the appropriate food concentration. Pairs were observed every second day for egg production and survival, when food and medium were changed. Females carrying eggs were isolated until their eggs hatched. Upon hatching, full-sib nauplii were separated into individual petrie dishes and raised to maturity on the low or high food concentrations described above. As described for the parental generation, we measured age and size at metamorphosis, and age and size at maturity for each offspring from each full-sib mating. After accounting for mortality and the low fecundity of some pairs, we had sufficient data to analyze for 12 families fed low food concentrations and 14 families raised on high food concentrations.

All copepods were fed a mixture of two algal species, *Cryptomonas erosa* and *Chlamydomonas reinhardtii*, that were cultured in modified MBL medium (Stemberger 1981) at 19°C and a photoperiod regime of 14 hr Light: 10 hr Dark. Concentrations of stock cultures were estimated daily using a hemacytometer and transformed to μg C/mL following the equations in Strathmann (1967). Appropriate volumes of each stock culture were then added to modified MBL medium to yield 0.1 μg C/mL of each species for low food conditions (total concentration = 0.2 μgC/mL) and 0.3 μg C/mL of each species for high food conditions (total concentration =

0.6 μg C/mL). Algal cultures were transferred to new medium weekly to maintain cultures in exponential growth phase.

Data Analyses

Summary statistics (means and standard deviations) for age and size at metamorphosis, age and size at maturity, larval growth rate (calculated as size at metamorphosis / length of larval period, which is an overestimate for all individuals because each was larger than 0 μm at birth), and juvenile growth rate ([size at maturity – size at metamorphosis] – [age at maturity – age at metamorphosis]) were calculated for each family raised at each food concentration. Spearman Rank correlations (age and size at metamorphosis occasionally were not normally distributed) were calculated between all trait pairs. Both phenotypic and genetic correlations were adjusted for the number of table-wise comparisons by revising α, the level of statistical significance (Rice 1989.

We estimated heritability in the broad sense ($h^2_B = V_G/V_P$) for each of these traits as described in Roff (1997); the required Mean Square values (variation within and among families) were obtained for each trait using one-way Analyses of Variance (ANOVA). Because ANOVA is robust to deviations for normality (Sokal and Rohlf 1995), we report all ANOVA results for untransformed data. Family sizes were not equal, and we calculated weighted mean family sizes (k; Roff 1997 p. 42) in order to estimate heritabilities. Because we used a full-sib mating design, our estimates of additive genetic variance are inflated by environmental variance between families (which should be small given our rearing design) and dominance effects (but see Simons

and Roff 1994). Standard errors for h^2 were estimated using Tukey's jackknife method (Roff and Preziosi 1994; Sokal and Rohlf 1995, p. 821-822) on untransformed values of the pseudovalues obtained from sequential h^2 estimates. For jackknife estimates, heritability was calculated repeatedly for both low and high food conditions, each time by removing one individual from the original data set. This procedure resulted in 80 jackknifed estimates of h^2 for low food conditions, and 118 estimates for high food conditions. We then used 1-tailed t-tests to identify h^2 values that were significantly greater than 0.

In the Low Food treatment, females were significantly larger ($F=26.61$, $p<0.0001$, $df=1, 55$) and older ($F=22.53$, $p<0.0001$, $df=1, 55$) than males; and among High Food individuals, size at maturity differed between sexes ($F=47.31$, $p<0.0001$, $df=1, 93$). As a result, we estimated heritabilities for size and age at maturity separately for both sexes. Although estimating heritabilities and genetic correlations (see below) for separate sexes provided more information, sample sizes were reduced and large standard errors of these estimates caused few of our estimates to be significantly different from zero. When ANOVA showed significant differences between sexes in traits, we report heritability estimates for each sex. When gender differences were not significant, we report estimates for combined individuals. Because we used female *D. leptopus* in the parental generation to determine the fitness consequences of several life history traits (Twombly and Tisch, submitted manuscript), we focus our interpretation of quantitative genetic parameters on this sex.

We estimated genetic correlations (r_G) between traits within an individual (Roff 1997) for the following pairs of traits: age and size at metamorphosis; age and size at maturity (sexes

separated); larval and juvenile growth rates; age at metamorphosis – age at maturity (sexes separated); size at metamorphosis – size at maturity (sexes separated), juvenile growth rate – size at maturity (sexes separated) and juvenile growth rate – age at maturity (sexes separated). For each correlation, the among-family variance in each trait was estimated independently using one-way ANOVA (Fry 1992); among family covariances were estimated using the CCSP H-matrix obtained from multivariate ANOVA (MANOVA). Tukey's jackknife method was used to estimate standard errors for all genetic correlations, after applying the z-transform to all estimates of each correlation (Sokal and Rohlf 1995, pp. 821-822). We used 2-tailed t-tests to identify genetic correlations that were significantly different from 0.

Results

In the parental generation, age at maturity (females only) correlated significantly with a composite measure of individual fitness, λ ($r=-0.457$, $p=0.0001$, $n=84$; Twombly and Tisch, submitted). None of the other traits we measured (size at metamorphosis, age at metamorphosis, size at maturity) correlated with fitness, but age at metamorphosis correlated significantly with age at maturity ($r=0.213$, $p=0.005$, $n=166$), suggesting that it also may contribute to fitness. These results showed that development (or age at molting, its proxy measure) is linked most closely with fitness and formed the basis for our prediction that heritability estimates for age (at metamorphosis and maturity) should be low.

Heritability estimates generally did not differ between treatments (Table 1); in both food conditions, age at metamorphosis, female age at maturity and larval growth rates exhibited levels

of additive genetic variation that were significantly different from zero. Size at maturity was significantly heritable only for males and only when raised on high food, and the remaining estimates were not significantly different from zero. In some instances, and particularly when sexes were analyzed separately, heritability values were substantial but standard error estimates were very large due to small sample sizes. Two of the traits with significant h^2 values (age at metamorphosis and age at maturity) were those that contributed significantly to fitness in the parental generation.

Phenotypic correlations: When adjusted for the number of pairwise comparisons made (Rice 1989), few phenotypic correlations were significant in either food treatment (Table 2). In both treatments, the first individuals to metamorphose were also the first to mature; this correlation was significant for females raised on high food and for males raised on low food. Larval and juvenile growth rates were not significantly related, suggesting that growth during the larval phase is independent of juvenile growth. Larval growth rate varied significantly with size and age at metamorphosis in both treatments (except for male size at high food). Larval growth rate was also correlated (negatively) with age at maturity in females raised on high food and in males raised on low food. In both food treatments, juveniles that grew fast metamorphosed early; at high food alone, juveniles that grew fast matured at larger sizes. Size and age at maturity were negatively, but not significantly, correlated. Development may be coupled between ontogenetic stages (age at metamorphosis and age at maturity are correlated), but the lack of a correlation between size at metamorphosis and size at maturity suggests that growth rate is free to respond to selection independently during different phases of the life cycle.

Genetic Correlations: Few of the genetic correlations shown in Table 3 are significantly different from zero. In both food treatments, age and size at metamorphosis were negatively and significantly correlated. Correlations between age and size at maturity were not significant for either sex in either treatment. Larval and juvenile growth rates were significantly correlated among males only. Among females, juvenile growth rates correlated negatively with age at maturity. Ontogenetic correlations (between size at metamorphosis and maturity or age at metamorphosis and maturity) were usually not significant indicating that selection can act independently on either age (development) or size (growth) in successive life cycle phases (Table 3). The one exception to this trend was that age at metamorphosis and age at maturity were significantly correlated for males raised at low food.

When h^2 values for particular traits are not significantly different from zero, estimates of genetic correlations involving these traits are hard to interpret (e.g., Berven 1987; Newman 1988). Our most meaningful estimates of genetic correlations are therefore for traits associated with development (significant h^2 for age at metamorphosis and maturity). These estimates show that development and growth were significantly correlated during the larval phase, and that the youngest individuals were the largest. Growth and development were not correlated among juveniles, however. Genetic correlations between larval and juvenile growth were significant for males only. While the same genes may regulate growth in both life cycle phases in males, our results suggest that selection can operate independently on larval and juvenile growth rates in females (the sex for which we measured fitness in a previous study). Larval and juvenile growth rates differed significantly in both high and low food treatments (Mann-Whitney Rank Sum test,

$p<0.001$), and both larval ($p=0.001$) and juvenile ($p<0.001$) growth rates were significantly lower under low food concentrations than in high food treatments.

Negative genetic correlations are considered some of the best evidence for the existence of numerous life-history tradeoffs predicted by theory (e.g., Reznick 1985; Roff 1992, but see Houle 1991). During the larval phase of *D. leptopus*, individuals that developed rapidly were smaller at metamorphosis than those that developed slowly, providing good evidence for a genetic tradeoff between larval growth and development. A similar tradeoff was not apparent during the juvenile phase, however, and a tradeoff between larval and juvenile growth rates was expressed only by males reared in high food concentrations.

Discussion

Evolutionary biologists commonly use estimates of heritability and genetic correlations to make inferences about how natural selection has worked on particular traits in the past or to reveal the potential for evolutionary change in the future. Our full-sib analysis of *D. leptopus* provided estimates of broad sense heritabilities, or additive genetic variation inflated by dominance, interaction and maternal effects. Although this measure overestimates the additive genetic component of variation (which is the basis for change due to selection), some previous studies have found the non-additive components of genetic variation to be small (e.g., Clayton et al. 1957; Mousseau and Roff 1987; Newman 1988) so that broad-sense measures may indicate levels of additive genetic variance (Simons and Roff 1994). At least in some cases, this substitution is not appropriate, however. Travis et al. (1987) found that dominance genetic

effects were large for hatchling size, growth rate, and length of the larval period in *Hyla crucifer*, and argued that dominance effects should be large for traits most directly connected to fitness.

Our results confirmed few of our initial predictions. Only age at metamorphosis, female age at maturity, and larval growth rates showed heritabilities significantly greater than zero; in the parental generation, the first two of these traits contributed most to individual fitness. These significant heritabilities indicate that selection has not eroded genetic variation for these traits and can most likely continue to modify developmental rates and ages at particular life-history transitions in this population of *Diaptomus leptopus*. Findings of substantial (additive) genetic variation for life-history or fitness traits are not unusual (e.g., Primack and Antonovics 1981; Price and Grant 1984; Berven 1987; Newman 1988), and there usually exists adequate variation for selection to act on these traits (e.g., Houle 1991). In fact, the potential for selection to act on fitness traits may exist even when heritability estimates are low, if the reason for low estimates is inflated environmental variance (e.g., Service and Rose 1985; Price and Schluter 1991). Specific predictions about the magnitude of heritabilities expected for life-history traits seem unrealistic (Price and Schluter 1991), and it may be more profitable to identify the processes that maintain this variation in the face of periodically strong directional selection. Demonstrated mechanisms include environmental heterogeneity or uncertainty (e.g., Rice 1987; Newman 1988; Mitchell-Olds 1992; Vavrek et al. 1996), mutation (Turelli 1984), antagonistic pleiotropy (e.g., Lande and Arnold 1983; Mitchell-Olds 1986, 1996), frequency dependence (e.g., Hori 1993) and migration (e.g., Bossart and Scriber 1995). The mechanism maintaining variation for development in *D. leptopus* is not known.

Also in contrast to our predictions, we found no significant genetic variation for size at metamorphosis or for size at maturity. In the parental generation, size or growth rates did not contribute to individual fitness and, following conventional wisdom, we expected to find relatively high levels of genetic variation for these traits. Our results suggest that body size in *D. leptopus* is unable to respond to selection in the field. There are at least four ways to interpret these results. First, body size appears to be constrained in this species (Twombly and Tisch 2000), and these constraints may reflect lower genetic variation and a limited ability to respond to selection. Second, low genetic variance is expected for fitness traits after strong selection has removed most existing genetic variation (Fisher 1958; Roff 1997). In natural zooplankton populations, a large emphasis is placed on the role that body size plays in mortality schedules (predation, e.g., Brooks and Dodson 1965), and the fitness consequences of body size may depend on natural mortality sources not revealed by our laboratory study (Twombly and Tisch, submitted). Third, genetic variation in freshwater crustaceans can be stored in a 'resting egg bank' (e.g., Weider et al. 1997; Hairston et al. 1999), so the amount of variation in body size (or age) measured in the active population at any one time may considerably underestimate the amounts of variation present. Finally, inflated environmental variance for body size due to a large number of environmental effectors (Price and Schluter 1991) or to exposure to a novel environment (Service and Rose 1985) may account for the low heritability estimates we obtained, and substantial genetic variation for body size or growth rates may in fact exist.

To what extent do quantitative genetics estimates made on laboratory populations (in 'novel' environments) apply to field populations? Our heritability estimates were by necessity based on laboratory-reared individuals, and their diet was limited compared with field diets.

Because quantitative genetics estimates are very sensitive to environmental conditions, the relevance of laboratory-derived estimates for field populations has been questioned. Holloway et al. (1990) found that increased genetic variance in novel (laboratory) environments resulted from the expression of genes not previously exposed to natural selection. As a further test of similarities between laboratory and field-derived estimates, Simons and Roff (1994) split sibships of the cricket, *Gryllus pennsylvanicus,* and raised sibs both in homogeneous laboratory environments and in the field. For all h^2 estimates except male and female development times, they recorded consistent and substantial reductions of h^2 in the more variable field environment. In a later study, these authors reported that translation of laboratory-derived genetic correlations to natural populations is also problematic. Only broad similarities were found between laboratory and field estimates of genetic correlations for life-history traits. Despite these results, Weigensberg and Roff (1996) claimed that levels of heritability typically measured for life-history traits under laboratory conditions are comparable to those measured in natural environments. These conflicting studies encourage caution in interpreting the estimates we report here and emphasize the importance of assessing the causes and consequences of size and age variation in field populations.

Hilbish and Vernberg (1987) estimated the genetic contribution to juvenile shape and growth in the mud crab, *Eurypanopeus depressus,* by partitioning phenotypic variation in these traits among and within families. They argued that significant variation among families in a trait implied that close relatives appear similar because they share the same genes. Using this approach, they found significant family effects (significant genetic variation) for growth rates over a number of successive juvenile molts, but not for body shape. Support for our findings of

lower genetic variation for body size (or growth) than for age (development) comes from variance component analyses of age and size at metamorphosis in several additional freshwater copepod species. For two populations of *Mesocyclops edax* , and one population each of *Cyclops vernalis, Diaptomus pygmaeus* ,and *D. sanguineus*, variance component analysis attributed 49% - 91% of the observed variation in age at metamorphosis to family effects (Twombly 1995). In agreement with the heritability results we present here, the family (genetic) contribution to intraspecific variation in size at metamorphosis was smaller and ranged from 12.5%-37.5%. Even though phenotypic variation may be exaggerated in laboratory-reared individuals, the similarities between variance component analyses and heritability measurements suggest that the general trend we have detected – that of more genetic variation for development than for growth – is correct.

Heritability estimates – and the genetic contribution to any trait – may change over time or with environment (e.g., Berven 1987; Platenkamp and Shaw 1992). These changes are also evident from variance component analyses of freshwater copepods. Laboratory-reared individuals of *Boeckella triarticulata* showed significant family effects for size at metamorphosis, maturity and death, but not for age (Twombly and Burns, unpublished data); and in *Cyclops scutifer,* the family (or genetic) contribution to age at metamorphosis changed over time. It was very low (8%) for a cohort hatched in June in Store Tryvann (Norway), but accounted for 60% of the age at metamorphosis variation in the August cohort (Twombly 1993). The results we report here reinforce previous conclusions that development, rather than growth, is the target of selection in field populations and that adequate additive genetic variation exists in natural populations for this trait to respond to selection.

Genetic correlations: Common in quantitative genetics analyses are the assumptions that 1) life-history traits, under strong directional selection, should exhibit negative genetic correlations (e.g., Roff 1996) and 2) negative genetic correlations constrain selection (Lande and Arnold 1983). These assumptions are questioned by the possibility that traits can co-evolve in ways not predicted by genetic covariance matrices (Houle 1991) and by the many positive genetic correlations estimated in field or laboratory populations (e.g., Mitchell-Olds 1986; Berven 1987; Bouin 1992; Platenkamp and Shaw 1992; Spitze 1995). These unexpected positive genetic correlations could be inaccurate representations of character interactions (Houle 1991) or they could result from exposure to novel environments (Holloway et al. 1990; Service and Rose 1985). Their commonness suggests that they are not spurious and that they may represent previously unexpected evolutionary dynamics (Reznick et al. 2000).

We based our original predictions for genetic correlations among life-history traits in *Diaptomus leptopus* on the assumption that they should in general be negative. Growth and development were negatively correlated during the larval phase in both high and low food conditions, indicating that individuals that were younger at metamorphosis were also larger. These correlations demonstrate that copepods do not maximize size at metamorphosis by delaying this transition, as has been repeatedly demonstrated for amphibians and insects (Blouin 1992; Berven 1987), and that these processes are not able to respond to selection independently during the larval phase. Our results indicate that this genetic correlation is broken at metamorphosis. Age and size at maturity were not genetically correlated in either treatment, suggesting that selection is free to act on each process individually. These results contradict

those based on phenotypic correlations (Table 2; Twombly and Tisch, submitted), but confirm Ebenman's (1992) proposal that metamorphosis breaks genetic correlations.

Because accurate estimates of genetic correlations are labor intensive (Roff 1996), and many studies (such as this one) are limited to sample sizes that, while practical, are statistically inadequate (e.g., Roach 1986), Roff (1996; Simons and Roff 1996) asked if phenotypic correlations could serve as a proxy for genetic correlations. In comparing genetic correlations between life-history, morphological, and behavioral traits in 51 non-domesticated species, Roff (1996) found a highly significant correlation between phenotypic and genetic correlations. This correspondence is, again, not uniformly reliable; we found only limited agreement between phenotypic and genetic correlations, as have others (e.g., Berven 1987).

One of the goals of our study was to address the question, for organisms with complex life cycles, of whether lifetime fitness entails compromises between correlated traits under selection in different environments. Genetic correlations between traits developed in any particular stage (habitat) may prevent subsequent stages from responding optimally to their environments. Among the many arguments proposed for the evolution of metamorphosis and complex life cycles, Ebenman (1992) suggested that metamorphosis breaks genetic correlations that develop between traits in a particular habitat. We know of few studies that have tested this hypothesis directly, even though estimates of heritabilities and genetic correlations are common. As evidence that evolution can act independently on larval and adult stages, Ebenman (1992) cited studies showing that diverse larval morphs metamorphose into similar adults, or vice versa. Other evidence gleaned from the literature is mixed. Bertram et al. (1993)demonstrated a

negative correlation between larval and juvenile growth in the winter flounder (compensatory growth), and Pechenik et al. (1996) documented the same pattern for *C. fornicata*. Both these organisms exhibit metamorphosis, but the data presented are phenotypic rather than genetic. Palmer and Dingle (1986) measured negative genetic correlations between larval and juvenile growth in the milkweed bug, *Oncopeltus,* and Roach (1986) found, for *Geranium carolinianum,* that traits advantageous at one stage (juveniles) were disadvantageous to adults. In contrast, Blouin (1992) found no significant genetic correlation between larval and juvenile growth rates in *Hyla cinerea.* In the harpacticoid copepod, *Tigriopus californicus,* genetic correlations for morphological characters were often stronger between traits within a stage than within a trait across stages (West, unpublished manuscript). These latter studies indicate that pre- and post-metamorphic body plans are at least partially decoupled genetically.

For *D. leptopus*, we also found little evidence for constraints or tradeoffs due to correlations among traits ontogenetically. The correlation between growth and development measured for larvae was broken at metamorphosis, and we found no evidence that particular traits (age or size) were correlated between successive ontogenetic stages. These data provide the strongest evidence to date that metamorphosis breaks genetic correlations, although laboratory-based estimates of genetic correlations need to be interpreted with some caution (Simons and Roff 1996). Although size and age appear free to evolve independently in juvenile *D. leptopus*, neither body size at metamorphosis or maturity appears to contribute to fitness in this species. Heritabilities for size at maturity differed from those estimated for size at metamorphosis and were generally high (but not significant due to large errors).

Understanding the significance of these heritability estimates and of the independence of development and growth among juvenile stages requires field studies to determine how predation and other environmental conditions affect size-specific mortality. The laboratory experiments we reported here provided information not available in field: individual variation in growth and development, contributions of these traits to individual fitness, and estimates of quantitative genetics parameters that form the basis for our prediction that selection acts most strongly on development. Although the evolutionary implications of quantitative genetic parameters estimated in laboratory environments may be difficult to interpret (Platenkamp and Shaw 1992; Simons and Roff 1996), small, mobile organisms like copepods cannot be marked and followed individually over time, precluding field estimates of these parameters. A complete understanding of life-history evolution in these abundant and ecologically important organisms therefore requires integration of the laboratory results we report here with future, field experiments.

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Table 1. Broad sense heritability estimates for *Diaptous leptopus*; standard errors are given in parentheses. Values in bold type are those significantly different from 0 (1-tailed t-test) based on α adjusted for the number of table-wise comparisons.

	low food	**high food**
size at metamorphosis	0.559 (0.267)	0.049 (0.154)
age at metamorphosis	**0.836 (0.308)**	**0.971 (0.161)**
age at maturity - females	**1.15 (0.271)**	**1.049 (0.229)**
males	0.409 (0.348)	-0.019 (0.224)
size at maturity - females	-0.042 (0.225)	0.644 (0.266)
males	0.566 (0.323)	**0.907 (0.228)**
larval growth rate	**0.858 (0.272)**	**0.927 (0.154)**
juvenile growth rate		
females	0.669 (0.309)	0.538 (0.256)
males	0.301 (0.293)	0.381 (0.326)

Table 2: Phenotypic Spearman Rank Correlations for life-history traits of F1 copepods raised at low (above the diagonal) and high (below the diagonal) food concentrations. A. Females; B. Males. n=number of individuals, smm = size at metamorphosis, amm = age at metamorphosis, sizemat = size at maturity, agemat = age at maturity, lgr = larval growth rate, jgr = juvenile growth rate. Correlations in bold are significant at the table-wise level of p=0.003.

A. Females: high food n=55; low food n=46;

	smm	amm	sizemat	agemat	lgr	jgr
smm		-0.390	0.056	-0.247	**0.562**	0.122
amm	-0.309		0.086	0.168	**-0.975**	0.075
sizemat	0.030	-0.132		-0.075	-0.074	0.247
agemat	**-0.474**	**0.529**	-0.054		-0.204	**-0.939**
lgr	**0.519**	**-0.964**	0.137	**-0.572**		-0.037
jgr	0.205	-0.035	**0.428**	**-0.746**	0.074	

B. Males: high food n=61; low food n=35

	smm	amm	sizemat	agemat	lgr	jgr
smm		**-0.469**	0.072	-0.189	**0.648**	0.028
amm	-0.037		0.180	**0.641**	**-0.962**	-0.415
sizemat	0.021	-0.098		0.318	-0.158	-0.179
agemat	0.053	0.178	-0.236		**-0.594**	**-0.925**
lgr	0.260	**-0.969**	0.082	-0.160		0.368
jgr	-0.045	0.209	**0.443**	**-0.834**	-0.215	

Table 3: Genetic correlations between trait pairs for *D. leptopus* raised at low food (above the diagonal) and high food (below the diagonal) conditions. A. Females; B. Males. n=number of individuals; other trait abbreviations are as in Table 2. Correlations in bold are significant at the tablewise level, p=0.003.

A. Females: high food n=55; low food n=46

	smm	amm	sizemat	agemat	lgr	jgr
smm		**-0.540**	0.033	---	---	---
amm	**-0.434**		---	0.358	---	---
sizeat	0.105	---		0.417	---	-0.443
agemat	---	0.201	-0.197		---	**-0.980**
lgr	---	---	---	---		0.032
jgr	---	---	0.401	**-0.773**	-0.045	

B. Males: high food n=61; low food n=35

	smm	amm	smat	amat	lgr	jgr
smm		**-0.540**	0.495	---	---	---
amm	**-0.434**		---	**0.887**	---	---
smat	0.099	---		0.533	---	-0.314
amat	---	-0.704	-0.114		---	0.943
lgr	---	---	---	---		**0.586**
jgr	---	---	0.485	-0.926	**-0.580**	