Correlated evolution of life-history with size at maturity in *Daphnia pulicaria*: patterns within and between populations

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Summary
Explaining the repeated evolution of similar sets of traits under similar environmental conditions is an important issue in evolutionary biology. The extreme alternative classes of explanations for correlated suites of traits are optimal adaptation and genetic constraint resulting from pleiotropy. Adaptive explanations presume that individual traits are free to evolve to their local optima and that convergent evolution represents particularly adaptive combinations of traits. Alternatively, if pleiotropy is strong and difficult to break, strong selection on one or a few particularly important characters would be expected to result in consistent correlated evolution of associated traits. If pleiotropy is common, we predict that the pattern of divergence among populations will consistently reflect the within-population genetic architecture. To test the idea that the multivariate life-history phenotype is largely a byproduct of strong selection on body size, we imposed divergent artificial selection on size at maturity upon two populations of the cladoceran *Daphnia pulicaria*, chosen on the basis of their extreme divergence in body size. Overall, the trajectory of divergence between the two natural populations did not differ from that predicted by the genetic architecture within each population. However, the pattern of correlated responses suggested the presence of strong pleiotropic constraints only for adult body size and not for other life-history traits. One trait, offspring size, appears to have evolved in a way different from that expected from the within-population genetic architecture and may be under stabilizing selection.

1. Introduction
It is a canon of evolutionary biology that organisms often evolve similar sets of traits under similar ecological circumstances (e.g. Corner, 1949; Berg, 1959; Gould, 1977; Houde & Endler, 1990; Barton & Dean, 1993; Cooper, 1995; Westneat, 1995; Robinson & Allgeyer, 1996; Ward & Seely, 1996; Ackerly & Donoghue, 1998; Armbruster *et al.*, 1999). There are two extreme alternative explanations for the consistent correlated evolution of suites of characters. First, it may be that particular combinations of traits are favoured by natural selection and that each trait independently evolves to its optimum value. Alternatively, there may be underlying biological relationships between traits (mechanical, physiological, energetic, etc.) that *invariably* require certain combinations of traits to co-occur and disallow the co-occurrence of other such combinations (Arnold, 1992).

In terms of the genetics underlying the traits in question, in the first case the traits must either be completely controlled by different loci or, if some loci have pleiotropic effects, there must be alleles that allow all possible combinations of trait values to co-occur. In this case, genetic correlations result from linkage disequilibrium maintained by natural selection. In the second case, at least some loci must have pleiotropic effects, and certain combinations of trait values must never occur, i.e. alleles that have a specific effect on some trait *A* must *invariably* affect some other trait *B* in a particular way. There have been many studies in recent years in which genetic correlations have been investigated, but relatively few have been designed in such a way as to discern between the two possible alternatives (but see Armbruster, 1991; Lynch & Spitze, 1994; Draye & Lints, 1995; Mitchell-Olds, 1996; Conner, 1997, Nuzhdin *et al.*,...
clearly genetic correlation at different hierarchical levels is inconsistent. In contrast, the observation of different patterns of life-history evolution among populations to among species. Conversely, if traits are substantially free to evolve independently from other traits, then the pattern of genetic correlation need not be consistent across different hierarchical levels. Accordingly, although the observation of consistent genetic correlations at all levels cannot rule out the possibility that the traits are in fact independent and that natural selection has just happened to favour that particular arrangement of traits, it is consistent with the presence of underlying pleiotropic constraints. In contrast, the observation of different patterns of genetic correlation at different hierarchical levels is clearly inconsistent with the presence of strong pleiotropic constraints (e.g. Lande, 1979; Arnold, 1992; Schluter, 1996).

The evolution of life-histories of freshwater zooplankton in the family Daphniidae provides a striking example of the consistent co-occurrence of suites of traits, typically associated with particular ecological circumstances (reviewed in Lynch, 1980). Daphnids occur in both permanent lakes and temporary ponds, and habitat shifts have occurred numerous times over the course of daphnid evolution (Colbourne et al., 1998; Taylor et al., 1999). In general, compared with temporary pond dwellers, species inhabiting permanent lakes tend to mature earlier and at smaller body sizes, produce smaller clutches of relatively larger (as a proportion of size at maturity) offspring, and invest more in adult growth (fig. 1 in Lynch, 1980). Substantial circumstantial evidence suggests that these different patterns of life-history evolution are driven by size-selective predation (Brooks & Dodson, 1965; Kerfoot, 1980; Kerfoot & Sih, 1987). Permanent lakes contain fish, which preferentially prey upon large individuals. In temporary ponds, the primary predators are often invertebrates, which are unable to consume large individuals. Selection can be extremely intense. For example, there are numerous well-documented cases of large-bodied species becoming extinct (Brooks & Dodson, 1965) or evolving behavioural changes (Cousyn et al., 2001) following the introduction of fish into permanent ponds. Divergence in life-history as a result of diversifying selection resulting from different predation regimes is not unique to daphnids; there are well-documented examples from a wide variety of taxa (e.g. Reznick et al., 1990; Wellborn, 1994; Martin, 1995).

Elucidation of the evolutionary mechanisms responsible for the divergence of Daphnia life-histories requires data on genetic covariation at multiple phylogenetic levels. Several studies report within-species genetic covariances (summarized in Lynch & Spitz, 1994; Spitz, 1995; Tessier & Leibold, 1997; Dudycha & Tessier, 1999), and data on multiple populations exist for two species, Daphnia obtusa (n=8) and Daphnia pulex (n=2) (Lynch & Spitz, 1994). For D. obtusa, the within-population genetic correlations generally reflect the among-population correlations, at least in sign, but the two populations of D. pulex did not differ in size at maturity and only very slightly in other traits (Lynch et al., 1989), so there is little information about among-population trends. Furthermore, in many cases the absolute values of genetic correlations were large but not statistically significant, due presumably to the low statistical power associated with only moderate sample sizes (Lynch & Walsh, 1998).

Here we more directly consider the relationships between size at maturity and other life-history traits in two populations of lake-dwelling Daphnia pulicaria by investigating the correlated response to artificial selection on size at maturity among clones. A correlated response to selection provides unambiguous evidence for the existence of a significant genetic correlation and of its sign. The motivation for this experiment was to evaluate whether genetic correlations with size at maturity within populations are consistent with the pattern of divergence between populations. This question is conceptually related to the commonly asked questions: ‘Is the genetic (co)variance matrix constant?’ (e.g. Lande, 1979; Shaw et al., 1995) and ‘Does evolution proceed along genetic lines of least resistance?’ (Schluter, 1996). The difference is that we are interested in the structure of a particular subset of the genetic covariance matrix (i.e. the column of covariances with size at maturity) for which we have strong a priori reason to believe may have evolved in a particular way.

2. Materials and methods

(i) Natural history of Daphnia pulicaria

Daphnia pulicaria is a planktonic cladoceran that occurs circumglobally in the Northern temperate...
zone. The two study populations were chosen to repre-
sent the extremes of body size found in *D. pulicaria*
in Oregon, based on a survey of 14 populations
throughout the state (K. Morgan & M. Pfrender, un-
published data). Individuals from Klamath Lake, in
the southern Cascades, are consistently large at ma-
turity (~2.05 mm (0.03 SE) in this study), whereas
individuals from Marie Lake, located near the Pacific
coast, are consistently small (~1.63 mm (0.01 SE),
this study). Several thousand animals were collected
from Klamath Lake in February 2000 by vertical
plankton haul using a Wisconsin net and returned to
the laboratory, where 600 adult females were placed
individually in 200 ml of lake water in clear plastic
cups kept on trays in a 20 °C walk-in incubator set to
a 12/12 h light/dark cycle. In a similar manner, ani-
mals were collected from Marie Lake in June 2000,
and again in July 2000, using the same protocol except
that fewer animals were taken in the July collection.
In each case, 50 to 100 vertical hauls were made from
a drifting canoe, so the collections should constitute a
representative sample of the genetic variation present
in each population.

(ii) Selection protocol

The goal was to select the 2.5% of clones with the
largest and smallest average lengths at maturity, eli-
minating the 95% of clones in the middle of the distri-
bution. Of the approximately 600 clones from each
population initially established in the experiment, 481
Klamath, 320 June Marie and 217 July Marie clones
survived to begin the selection protocol. For logistical
reasons, the Klamath and June Marie collections were
divided into blocks of approximately 100 animals
each. Prior to the initiation of the selection exper-
iment, each clone was taken through two generations
of asexual reproduction to remove maternal effects.
Two days after release of a second-generation fe-
male’s third (occasionally second or fourth) clutch, a
single haphazardly chosen offspring from each clone
was placed in a cup containing 150 ml of filtered aged
tap water and approximately 3 × 10^5 cells/ml of the
green algae *Scenedesmus* sp. as food and raised to
maturity. Upon appearance of an individual’s first
clutch, that individual was measured using an ocular
micrometer at × 50 on a Wild M8 dissecting micro-
scope. The largest approximately 20% of animals in
each block were assigned to the High treatment and
the smallest approximately 20% were assigned to the
Low treatment. The first two clutches of each surviv-
ing animal were then discarded, and four offspring
from the third (occasionally second or fourth) clutch
were established individually under the same con-
ditions as before and measured at maturity. Average
size at maturity was calculated for each clone, and
the largest 50% of clones in the High treatment and
the smallest 50% of clones in the Low treatment were
kept for the next generation. After this second gener-
ation of selection, two third-clutch offspring from
each individual were kept, raised to maturity and
measured; again, the average adult size of the off-
spring of each selected mother was obtained, and the
half of the clones with the largest (smallest) average
adult size were kept for the next generation. Selection
proceeded in this way, with the number of clones be-
ing halved and the number of mothers per clone being
doubled each generation, until two clones from each
treatment remained in each block in the Klamath (five
blocks) and June Marie (three blocks) experiments,
and five clones from each treatment remained for the
July Marie experiment.

In principle, clonal selection need not be carried out
for multiple asexual generations. If the heritability of
the selected trait is 1, no additional benefit is gained
from increasing the number of individuals per clone
after the first asexual generation. However, if herita-
bility is very low, the optimum selection regime in-
volves culling only a small number of the most
extreme clones and continuing for many asexual gen-
erations, because individual phenotype will be a poor
predictor of genotypic value. For intermediate heri-
tabilties, there is a trade-off between number of in-
dividuals per clone and selection intensity (the frac-
tion of clones kept per asexual generation), analogous
to the trade-off between number of offspring per
family and number of families in a classical breeding
design to estimate components of genetic variance
(Lynch & Walsh, 1998). As the number of clones is
progressively reduced, so is the among-clone variance,
necessitating a concomitantly larger number of in-
dividuals per clone to detect a real difference between
clones.

(iii) Life table

Upon completion of the selection experiment, life-
history data were collected using standard life-table
analysis (Lynch et al., 1989) of the selected clones
(10 High and 10 Low from each population). Briefly,
prior to the assay 12 replicate lines were established
from each clone by use of single juvenile females
placed individually in cups containing 150 ml filtered
aged tap water and approximately 3 × 10^5 cells/ml
*Scenedesmus*. The water was changed every second
day, and trays of cups were rotated systematically to
minimize incubator position effects. Each replicate
was maintained in this manner for three generations
to minimize the effects of unique environments, and a
single female offspring from each third-generation
individual’s second clutch was isolated for life-table
analysis.

Individuals were observed every second day except
between release of the second and third clutch, at
which point they were observed daily. Life-history characters recorded were: length at maturity \((L_m)\), with maturity being defined by the appearance of eggs in the brood chamber; length at release of the third clutch \((L_3)\); number of offspring in the second clutch \((C_2)\); average number of offspring in the third and fourth clutches \((C_{3,4})\); average length of five newborn offspring in the third (and occasionally the fourth) clutch \((L_{3b})\); age at maturity \((k_m)\); interval between clutches, averaged over the first three clutches \((\text{INT}_{13})\); juvenile growth rate, calculated as \(G_j = \log(L_m/L_{3b})/t\), where \(t\) is the time between the two points adjusted as described below; and adult growth rate, calculated as \(G_a = \log(L_3/L_1)/t\), where \(L_1\) is length at release of the first clutch, \(L_3\) is length at release of the third clutch, and \(t\) is the adjusted time between the two points. These variables were chosen to allow comparison with the data summarized in table 6.3 of Lynch & Spitze (1994). We do not consider size of the first clutch because Klamath animals frequently aborted the first clutch and we consider a first clutch size of zero as misleading with respect to maternal investment in reproduction; the tendency to abort disappeared at subsequent clutches.

To improve the accuracy of estimated ages at reproduction, the developmental stages of eggs observed in the brood chamber were assigned to one of 12 categories based on embryonic morphology (Lynch et al., 1989), and the relative durations of these stages were estimated from the distribution of observed data. Thus, rather than recording ages at which clutches were released in daily intervals, more precise estimates were made by subtracting the expected time to reach the embryonic stage in a developing clutch carried by a mother at the time of observation from the time at which a released clutch was first observed. This refinement is made possible by the fact that Daphnia typically extrude a new clutch into the brood chamber almost immediately after releasing a clutch.

(iv) Data analysis

The experiment was analysed following the ‘genetic lines of least resistance’ approach of Schluter (1996), with the mathematical details following Pimentel (1979). The trajectory (vector) of the divergence between two groups \(i\) and \(j\), \(z\), is calculated as \(z = [X_i - X_j][X_i - X_j]^T(X_i - X_j)]^{-1/2}\), where \(X_i\) and \(X_j\) are the vectors of trait means of groups \(i\) and \(j\), respectively, and \(^T\) represents matrix transposition. This calculation scales the trajectory of divergence so that \(z^Tz = 1\) and is conceptually equivalent to transforming a covariance into a correlation. Population means were calculated as the unweighted mean of all High and Low lines. ANOVA revealed no significant effects of collection date in the Marie sample, so the Marie data were pooled across collection dates. The coefficients \(z\) of the vector \(z\) represent the contribution of each phenotypic trait \(x\) to the overall trajectory of divergence between the two groups. Traits with coefficients of the same sign diverge in the same general direction, i.e. an increase in one trait is associated with an increase in a second trait, whereas traits with coefficients of opposite signs diverge in opposite directions. The magnitude of \(z\), reflects the relative magnitude of the divergence between the groups. In the case of selected lines, the vector coefficients represent the response to selection, either direct (for the trait under direct selection) or correlated. Two trajectories of divergence, \(z_i\) and \(z_j\), can be compared in terms of the angle \(\theta\) between the two vectors, calculated as \(\theta = \cos^{-1}[z_i^Tz_j]\). If the two trajectories are identical, \(\theta = 0\); if the two trajectories are exactly opposite in all dimensions, \(\theta = \pi\) radians (180°).

To test the hypothesis that a vector \(z_i\) calculated from experimental data does not differ from an arbitrary pre-specified vector \(z_s\), we can resample the data, recalculate the vector (call it \(z_i^\star\)), and calculate the angle \(\theta^\star\) between the new vector \(z_i^\star\) and the original vector \(z_i\) (not \(z_s\)). The fraction of bootstrap iterations in which \(\theta^\star\) exceeds the original angle \(\theta\) between \(z_i\) and \(z_s\) is the approximate \(P\) value for the test of the hypothesis that \(z_i\) does not differ from \(z_s\) (Schluter, 1996; Efron & Tibshirani, 1986). We report the hypothesis test as \(P(\theta = 0)\) for conceptual clarity, but we emphasize that the estimated value of \(\theta\) will always be greater than zero because zero is the asymptotic lower bound for \(\theta\).
Correlated evolution in Daphnia

The respective contributions to the vectors \( z_1 \) and \( z_2 \) of individual phenotypic traits \( x \) can be compared by comparing the vector coefficients \( z_{11} \) and \( z_{22} \). Resampling can provide confidence intervals for \( z_{11} \) and \( z_{22} \).

Our primary question of interest involves the comparison of the trajectories of divergence between the High and Low selected lines within each population with the trajectory of divergence between the two populations (Fig. 1). We will refer to the trajectories of divergence between selected lines within Marie and Klamath as \( z_M \) and \( z_K \), respectively, and the trajectory of divergence between the two populations as \( z_{MK} \).

The angle between \( z_M \) and \( z_{MK} \) will be called \( \theta_M \), the angle between \( z_K \) and \( z_{MK} \) will be called \( \theta_K \), and the angle between the two within-population trajectories \( z_M \) and \( z_K \) will be called \( \theta_{MK} \). Hypothesis tests and confidence intervals on vector coefficients were determined by bootstrapping over clone means; this test assumes that family means were calculated without error. Statistical significance of vector coefficients was determined by the fraction of 2000 bootstrap replicates falling outside the desired confidence limit (e.g. for a desired two-tailed \( P < 0.05 \), a result is considered significant if \( < 2.5\% \) of bootstrap replicates were smaller (larger) than the observed value).

Broad-sense heritabilities \( (H^2) \) and coefficients of genetic variation \( (CV_G) \) were calculated as the among-clone component of variance of the trait; \( CV_G \) was calculated as the square-root of the among-clone component of genetic variance divided by the population phenotypic mean.

### Table 1. Mean values of life-history characters, averaged over all clones in that hierarchical level

<table>
<thead>
<tr>
<th>Pop</th>
<th>Tr</th>
<th>( L_{ab} )</th>
<th>( L_m )</th>
<th>( L_3 )</th>
<th>( C_2 )</th>
<th>( C_{3,4} )</th>
<th>( k_m )</th>
<th>( \text{INT}_{13} )</th>
<th>( H )</th>
<th>( G_j )</th>
<th>( G_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marie</td>
<td>Both</td>
<td>0.779</td>
<td>1.627</td>
<td>2.019</td>
<td>6.590</td>
<td>8.526</td>
<td>6.330</td>
<td>3.088</td>
<td>0.125</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.031</td>
<td>0.008</td>
<td>0.013</td>
<td>0.155</td>
<td>0.032</td>
<td>0.346</td>
<td>0.115</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.819</td>
<td>1.650</td>
<td>2.050</td>
<td>6.523</td>
<td>8.263</td>
<td>6.332</td>
<td>3.144</td>
<td>0.125</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.063</td>
<td>0.012</td>
<td>0.020</td>
<td>0.259</td>
<td>0.544</td>
<td>0.119</td>
<td>0.048</td>
<td>0.003</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.738</td>
<td>1.605</td>
<td>1.988</td>
<td>6.657</td>
<td>8.786</td>
<td>6.335</td>
<td>3.063</td>
<td>0.125</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003</td>
<td>0.007</td>
<td>0.012</td>
<td>0.197</td>
<td>0.307</td>
<td>0.103</td>
<td>0.019</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Klamath</td>
<td>Both</td>
<td>0.743</td>
<td>2.048</td>
<td>2.714</td>
<td>9.553</td>
<td>19.764</td>
<td>7.164</td>
<td>2.975</td>
<td>0.143</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.007</td>
<td>0.027</td>
<td>0.042</td>
<td>0.860</td>
<td>1.072</td>
<td>0.088</td>
<td>0.041</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.764</td>
<td>2.139</td>
<td>2.862</td>
<td>9.681</td>
<td>20.771</td>
<td>7.211</td>
<td>3.020</td>
<td>0.144</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.009</td>
<td>0.023</td>
<td>0.029</td>
<td>0.726</td>
<td>0.813</td>
<td>0.068</td>
<td>0.060</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.722</td>
<td>1.958</td>
<td>2.565</td>
<td>9.425</td>
<td>18.31</td>
<td>7.118</td>
<td>2.930</td>
<td>0.142</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006</td>
<td>0.027</td>
<td>0.042</td>
<td>0.662</td>
<td>2.023</td>
<td>0.172</td>
<td>0.057</td>
<td>0.004</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Standard errors are reported below means. Pop, population; Tr, treatment; \( L_{ab} \), length (mm) at birth; \( L_m \), length at maturity; \( L_3 \), length at clutch 3; \( C_2 \), size of second clutch; \( C_{3,4} \), average size of third and fourth clutches; \( k_m \), age at maturity (days); \( \text{INT}_{13} \), average interval between clutches (days); \( G_j \), juvenile growth rate; \( G_a \), adult growth rate.

### Table 2. Broad-sense heritabilities \( (H^2) \) and coefficients of genetic variation \( (CV_G) \) for life-history characters

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marie</th>
<th>Klamath</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_{ab} )</td>
<td>0.377</td>
<td>0.037****</td>
</tr>
<tr>
<td>( L_m )</td>
<td>0.423</td>
<td>0.079****</td>
</tr>
<tr>
<td>( L_3 )</td>
<td>0.655</td>
<td>0.078****</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>0.024</td>
<td>0.027</td>
</tr>
<tr>
<td>( C_{3,4} )</td>
<td>0.135</td>
<td>0.068****</td>
</tr>
<tr>
<td>( k_m )</td>
<td>0.017</td>
<td>0.012</td>
</tr>
<tr>
<td>( \text{INT}_{13} )</td>
<td>0.02</td>
<td>0.026</td>
</tr>
<tr>
<td>( G_j )</td>
<td>0.024</td>
<td>0.037</td>
</tr>
<tr>
<td>( G_a )</td>
<td>0.156</td>
<td>0.130****</td>
</tr>
</tbody>
</table>

Trait abbreviations are as in Table 1. Superscripted values are significantly greater than zero at the designated level. See Section 2 for details of calculations. **** \( P < 0.0001 \); * \( 0.05 < P < 0.01 \).

Neither calculation distinguishes between within- and between-treatment variance. Variance components were calculated by restricted maximum likelihood (REML) in the VARCOMP procedure of SAS version 8.8 for each population separately. Statistical significance of the resulting variance components was assessed as the effect of “clone” in one-way ANOVA performed with the GLM procedure of SAS.

### 3. Results

All results are summarized in Table 1 (trait means), Table 2 (broad-sense heritabilities and coefficients of
Each $r_{ij}$ (for example, note that the CVG of size at birth in due in large part to the much larger measurement er-
heritabilities of time-dependent traits are probably
than is body size or number of offspring, so the lower
lease) is much more difficult to measure accurately
of life-history events (birth, maturation, clutch re-
interval) had much lower heritabilities in both
populations than did body size or clutch size. Timing
of life-history events (birth, maturation, clutch re-
lease) is much more difficult to measure accurately
than is body size or number of offspring, so the lower
heritabilities of time-dependent traits are probably
due in large part to the much larger measurement er-
for example, note that the CVG of size at birth in
Marie is only slightly greater than the CVG of juve-
nile growth rate in Klamath (0.037 vs 0.030), yet the
heritability for the former is an order of magnitude
larger than the latter (0.377 vs 0.037), a result that
strongly suggests the lower heritability is due simply
to greater environmental variance rather than lower
heritability (Houle, 1992)). Inter-clutch interval
may be an exception to this claim, although we did
detect a small but significant difference between the
population means.

Both populations responded directly to clonal
selection in the expected way, i.e. High clones were
significantly larger at maturity than Low clones
($P < 0.0005$). The overall trajectories between selected
lines within each population did not differ signifi-
cantly from the trajectory of divergence between the
two populations ($\Pr(\theta_M = 0) > 0.20; \Pr(\theta_K = 0) > 0.48$).
Interpretation of the comparison of the trajectories of
divergence within each population with each other
($\theta_{MK}$) is less straightforward. With infinite data,
arbitrarily holding one trajectory (vector) fixed at
the observed value and resampling the data from the
other population will produce the same result irres-
pective of which population is resampled. With finite
data, the results need not be identical because the
phenotypic variation within each population will af-
fect the bootstrap distributions of $\theta_M$ and $\theta_K$. When
$\theta_M$ is held constant and compared with the bootstrap
distribution of $\theta_K$, $\Pr(\theta_{MK} = 0) > 0.13$; when $\theta_K$ is
held constant and compared with the bootstrap dis-
bution of $\theta_M$, $\Pr(\theta_{MK} = 0) > 0.38$. Thus, we cannot
reject the hypothesis that $z_M = z_K$, but the strength
of the inference differs depending on which trajectory is
arbitrarily held constant.

Each $z_i$ represents the contribution of phenotypic trait $x_i$ to the overall divergence between the two groups; $z_{x,MK}$ is the comparison between the two populations, $z_{x,M}$ is the comparison between High and Low selected lines in Marie and $z_{x,K}$ is the comparison between selected lines in Klamath. Rows are phenotypic traits; $L_m$ is the trait under direct selection. Values in parentheses are 95% bootstrap confidence intervals; values that are significantly different from 0 are in bold type. Traits superscripted with an $H$ are significantly heritable within that population. See Section 2 for details of calculations and interpretation. Trait abbreviations are as in Table 1.

### Table 3. Vector coefficients $z_{x,i}$ of $z_x$, the trajectory of divergence between two groups

<table>
<thead>
<tr>
<th>Trait $x$</th>
<th>$z_{x,MK}$</th>
<th>$z_{x,M}$</th>
<th>$z_{x,K}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_m$</td>
<td>0.166</td>
<td>0.297$^H$</td>
<td>0.240$^H$</td>
</tr>
<tr>
<td></td>
<td>(0.135, 0.211)</td>
<td>(0.091, 0.537)</td>
<td>(0.089, 0.528)</td>
</tr>
<tr>
<td>$L_a$</td>
<td>0.00026</td>
<td>0.184$^H$</td>
<td>0.161$^H$</td>
</tr>
<tr>
<td></td>
<td>(-0.015, 0.016)</td>
<td>(0.030, 0.395)</td>
<td>(0.047, 0.372)</td>
</tr>
<tr>
<td>$L_g$</td>
<td>0.211</td>
<td>0.377$^H$</td>
<td>0.294$^H$</td>
</tr>
<tr>
<td></td>
<td>(0.174, 0.263)</td>
<td>(0.101, 0.688)</td>
<td>(0.119, 0.160)</td>
</tr>
<tr>
<td>$C_a$</td>
<td>0.178</td>
<td>0.106</td>
<td>0.232$^H$</td>
</tr>
<tr>
<td></td>
<td>(0.019, 0.292)</td>
<td>(-0.547, 0.637)</td>
<td>(-0.742, 0.764)</td>
</tr>
<tr>
<td>$C_{a,4}$</td>
<td>0.939</td>
<td>-0.034$^H$</td>
<td>0.621$^H$</td>
</tr>
<tr>
<td></td>
<td>(0.911, 0.959)</td>
<td>(-0.975, 0.951)</td>
<td>(-0.597, 0.961)</td>
</tr>
<tr>
<td>$k_{\text{m}}$</td>
<td>0.087</td>
<td>0.084</td>
<td>0.049$^H$</td>
</tr>
<tr>
<td></td>
<td>(0.058, 0.123)</td>
<td>(-0.235, 0.416)</td>
<td>(-0.078, 0.258)</td>
</tr>
<tr>
<td>$\text{INT}_{\text{m}}$</td>
<td>-0.032</td>
<td>-0.0518</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>(-0.055, -0.011)</td>
<td>(-0.326, 0.146)</td>
<td>(-0.013, 0.338)</td>
</tr>
<tr>
<td>$G_j$</td>
<td>0.013</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>(0.010, 0.017)</td>
<td>(-0.038, 0.055)</td>
<td>(-0.019, 0.022)</td>
</tr>
<tr>
<td>$G_a$</td>
<td>0.005</td>
<td>0.014$^H$</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>(0.004, 0.007)</td>
<td>(-0.000, 0.035)</td>
<td>(-0.010, 0.007)</td>
</tr>
</tbody>
</table>

All body-size traits were highly heritable, as were
clutch sizes, with the exception of second clutch size in
Marie. The Marie population suffered considerable
mortality in the transition from the field to the lab-
oralatory, but genetic variation for most traits was not
much lower in the Marie sample than in the Klama-
th sample (average CVG for Marie = 0.051, for Kla-
th = 0.049), so it seems unlikely that the Marie
sample was purged of variation as a byproduct of
strong viability selection. Most traits with a time-
component (age at maturity, growth rates, inter-
clutch interval) had much lower heritabilities in both
populations than did body size or clutch size. Timing
of life-history events (birth, maturation, clutch re-
lease) is much more difficult to measure accurately
than is body size or number of offspring, so the lower
heritabilities of time-dependent traits are probably
due in large part to the much larger measurement er-
for example, note that the CVG of size at birth in
Marie is only slightly greater than the CVG of juve-
nile growth rate in Klamath (0.037 vs 0.030), yet the
heritability for the former is an order of magnitude
larger than the latter (0.377 vs 0.037), a result that
strongly suggests the lower heritability is due simply
to greater environmental variance rather than lower
heritability (Houle, 1992)). Inter-clutch interval
We next consider the contributions of individual phenotypic traits to the overall divergence between groups. For eight of nine traits, the trajectory of divergence between selected lines within each population did not differ significantly from the trajectory between populations, as evidenced by overlap of the 95% confidence limits of $(1) z_{x,M}$ and $z_{x,K}$ and $(2) z_{x,K}$ and $z_{x,M,K}$ (Table 2). Only size at birth (abbreviated $L_{48}$) differed between the two categories of comparisons (between selected lines within populations and between populations): the two populations did not differ in average size at birth, but in both populations animals from High lines were consistently born larger than those from Low lines. For all nine traits the response to selection in Klamath did not differ from the response to selection in Marie, again as evidenced by the overlap of the 95% confidence limits of $z_{x,M}$ and $z_{x,K}$ (Table 2). Although the statistical validity of comparing individual vector coefficients when the overall trajectories do not differ may be questioned (analogous to performing post hoc comparisons when an effect in an ANOVA is not significant), we may alternatively adopt the viewpoint that each comparison of $z_{x}$ between groups is a separate hypothesis test, subject to the (conservative) Bonferroni criterion of experiment-wide significance $\alpha = 0.05$. Taking this approach, the level of significance for each $z_{x}$ is $0.05/9 = 0.00056$, where 9 is the number of traits considered. For the Klamath population, there was no overlap between the bootstrap distributions of the within-population vector coefficient for size at birth ($z_{L,B.K}$) and the among-population vector coefficient ($z_{L,B,K,M}$). For the Marie population, approximately 1.5% of bootstrap replicates of $z_{L,B,M}$ were within the upper 99.5% confidence interval of $z_{L,B,K,M}$, which we interpret as a marginally significant difference.

4. Discussion

The two populations represented here, chosen on the basis of their extreme divergence in size at maturity, have also diverged significantly in almost every aspect of life-history examined in this study (Tables 1, 3). Moreover, the trajectories are consistently positive – reflected the general among-species pattern wherein small species typically have relatively larger offspring (i.e. as a fraction of adult body size) than do large species (Lynch, 1980).

Our experiment differed from a typical selection experiment in that it was designed to segregate out the most extreme genotypes across asexually reproducing generations within a single sexually reproducing generation rather than generate a response across sexually reproducing generations. This means that (1) non-additive genetic variance could contribute to the observed divergence among clones, (2) upon sexual
reproduction, recombination would potentially break up linkage disequilibrium generated by selection, causing a reversion toward the mean phenotype (Deng & Lynch, 1996), and (3) there is no information about the relationships between traits at phenotypic scales beyond those observed within each population. Obviously, it would be desirable to select across sexual generations as well as within them. Unfortunately, the inherent complexity of *Daphnia* reproductive biology (inducing production of males, hatching ephippia) makes cross-generational selection impractical. However, natural selection among clones is demonstrably important in cladocerans, particularly for species living in permanent lakes (Hebert, 1974; Lynch, 1987; Spitze, 1991). Our experiment was designed to reveal what is left after a single panmictic gene pool is subjected to very strong diversifying selection, which we believe mimics a biologically relevant situation.

Given the relative consistency of the within- and between-population trajectories of divergence, one obvious potential explanation for our failure to detect correlated responses to selection is insufficient statistical power. A much larger experiment would probably have enabled us to detect a modest difference in the within- and between-population trajectories. However, there are two reasons to think that the absence of correlated responses is genuine. First, when we reduced the among-population bootstrap sample size to that of the among-treatment, within-population sample size (*n* = 10), the confidence limits on the between-population vector coefficients (\(z_{KM}\)) increased only on the order of 30%, not nearly enough to explain the much greater variability in the within-population vector coefficients. Second, recall that we reduced a sample of approximately 500 randomly chosen clones to 10 extremely large (small) clones within each population. This is very intense diversifying selection and the direct responses to selection in each population were highly significant (completely non-overlapping bootstrap distributions of \(z_{Sm}\)), as were the correlated responses to selection for body size at other ontogenetic stages (Table 2). Any genetic correlation sufficiently weak to be undetectable with this degree of response to direct selection is not likely to constitute an important long-term constraint to phenotypic evolution. Although we cannot say with certainty that our 10 largest (smallest) clones were in fact the actual 10 largest (smallest), simulations of the selection process suggest that our design was very close to the optimal design for traits withheritabilities on the order of 20% (Ricardo Alía, unpublished results).

Ultimately, our interest is in the nature of the genetic variation underlying the traits observed in this study, both within and between populations. The nature of the factors responsible for segregating variation within populations and the relationship between them and the factors responsible for the divergence among populations is an unresolved (and contentious) issue (e.g. Orr, 2001; Barton & Keightley, 2002). For example, it remains to be determined whether most segregating variation within populations results from slightly deleterious alleles at mutation – selection balance or from alleles maintained at intermediate frequency by natural selection (Lande, 1976; Turelli, 1984; Gillespie & Turelli, 1989; Charlesworth & Hughes, 2000). The few available data reveal that mutational correlations between life-history traits are usually large and positive (Houle et al., 1994; Camara & Pigliucci, 1999; Keightley et al., 2000), although it is not known to what extent those correlations reflect pleiotropic effects (see Keightley et al., 2000). Were we to assume that the segregating variation within the two populations in this study is solely the product of recurrent slightly deleterious mutations, we must conclude that alleles that affect body size either do not, on average, have strong pleiotropic effects on other life-history traits, or if they do, that those effects are variable (e.g. some alleles that reduce body size pleiotropically decrease clutch size and some alleles that reduce body size pleiotropically increase clutch size). Alternatively, it may be that new, slightly deleterious mutations do have consistent pleiotropic effects, in which case segregating variation in these populations is actively maintained by some sort of balancing selection.

There has been much speculation that the alleles underlying phenotypic divergence among populations and higher taxa are, collectively, in some way qualitatively different from those responsible for segregating variation within populations (e.g. Gould, 1977; Orr & Coyne, 1992; Haag & True, 2001; Orr, 2001). The results from this study offer scant support for that notion, at least in terms of effects on the suite of traits in question. For example, if we wanted to find an animal that matured at large body size and had large clutches, long juvenile development time and high adult growth rate, we could go into either population and pick one off the shelf, so to speak (i.e. it would not be statistically improbable to find such an animal). Likewise, we could also find a large animal with the same suite of attributes except that it produces small clutches (or matures early, etc.). The only pleiotropic constraint on our phenotypic shopping affects body size at different ontogenetic stages: we would be unable to find an animal large at maturity that is small at the time it releases its third clutch.

There is an important caveat to our claim that pleiotropic constraints are largely unimportant: pleiotropic effects may depend on the magnitude of the phenotypic change. For example, a very small reduction in body size may have no effect on number of offspring whereas a large decrease in body size may necessitate a reduction in the number of offspring due
to space constraints (or, viewed another way, a large increase in body size could allow an increase in off-spring number, thereby alleviating a pre-existing constraint). Thus, the failure to observe a consistent pattern of constraint with small changes in phenotype may not rule out the presence of a constraint over larger distances in phenotypic space. Detailed understanding of the evolution of the multivariate phenotype of any organism will ultimately require knowledge of the pattern of genetic correlations over the full range of phenotypic and phylogenetic space.

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