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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.*,

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1999). The salient point is this: in the absence of extremely detailed genetic information (i.e. at the level of the actual gene(s) encoding the phenotypic trait), it is not possible to discriminate definitively between pleiotropy and linkage disequilibrium as the underlying cause of a genetic correlation *within a single population*, nor can the cause of divergence between populations be definitively identified. However, if a genetic correlation is the result of linkage disequilibrium maintained by selection, the within- and among-population correlations will be the same only if multivariate selection is the same within and among populations.

The two alternative hypotheses for the evolution of correlated traits lead to a specific set of predictions. If suites of characters are constrained by unbreakable (or difficult to break) pleiotropic relationships, then the pattern of genetic correlation should be consistent across a range of hierarchical levels, from within 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 & Spitze, 1994; Spitze, 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 & Spitze, 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

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zone. The two study populations were chosen to represent 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, unpublished data). Individuals from Klamath Lake, in the southern Cascades, are consistently large at maturity (~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, animals 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, eliminating the 95% of clones in the middle of the distribution. 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 experiment, each clone was taken through two generations of asexual reproduction to remove maternal effects.

Two days after release of a second-generation female'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 $\times 50$ on a Wild M8 dissecting microscope. 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 surviving animal were then discarded, and four offspring from the third (occasionally second or fourth) clutch were established individually under the same conditions 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 generation of selection, two third-clutch offspring from each individual were kept, raised to maturity and measured; again, the average adult size of the offspring 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 being 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 heritability is very low, the optimum selection regime involves culling only a small number of the most extreme clones and continuing for many asexual generations, because individual phenotype will be a poor predictor of genotypic value. For intermediate heritabilities, there is a trade-off between number of individuals per clone and selection intensity (the fraction 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 individuals 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_{03}); age at maturity (k_m); interval between clutches, averaged over the first three clutches (INT_{13}); juvenile growth rate, calculated as $G_j = \log(L_m/L_{03})/t$, 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, $\mathbf{z}$, is calculated as $\mathbf{z} = [\mathbf{X}_i - \mathbf{X}_j][(\mathbf{X}_i - \mathbf{X}_j)^T(\mathbf{X}_i - \mathbf{X}_j)]^{-1/2}$, where $\mathbf{X}_i$ and $\mathbf{X}_j$ 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 $\mathbf{z}^T\mathbf{z} = 1$ 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_x of the vector $\mathbf{z}$ 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_x 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, $\mathbf{z}_1$ and $\mathbf{z}_2$, can be compared in terms of the angle θ between the two vectors, calculated as $\theta = \cos^{-1}[\mathbf{z}_1^T\mathbf{z}_2]$. If the two trajectories are identical, $\theta = 0$; if the two trajectories are exactly opposite in all dimensions, $\theta = \pi$ radians (180°).

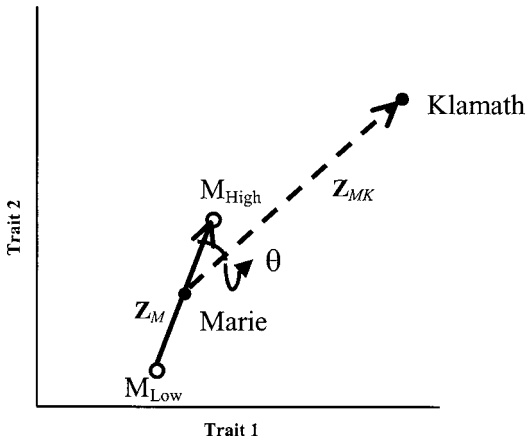

Fig. 1. Trajectories of phenotypic divergence between groups, depicted in two dimensions. Filled circles represent phenotypic means for the two populations; empty circles represent means of the Marie High and Low lines. The dashed line $\mathbf{z}_{MK}$ is the trajectory of divergence between the two base populations. The continuous line $\mathbf{z}_M$ is the trajectory of divergence between High (M_{High}) and Low (M_{Low}) selected lines in the Marie population. θ is the angle of divergence between the two trajectories. See Section 2 for details of calculations and interpretation.

To test the hypothesis that a vector $\mathbf{z}_1$ calculated from experimental data does not differ from an arbitrary pre-specified vector $\mathbf{z}_2$, we can resample the data, recalculate the vector (call it $\mathbf{z}_1^*$), and calculate the angle θ^* between the new vector $\mathbf{z}_1^*$ and the original vector $\mathbf{z}_1$ (*not* $\mathbf{z}_2$!). The fraction of bootstrap iterations in which θ^* exceeds the original angle θ between $\mathbf{z}_1$ and $\mathbf{z}_2$ is the approximate P value for the test of the hypothesis that $\mathbf{z}_1$ does not differ from $\mathbf{z}_2$ (Schluter, 1996; Efron & Tibshirani, 1986). We report the hypothesis test as $\Pr(\theta = 0)$ for conceptual clarity, but we emphasize that the *estimated* value of θ will always be greater than zero because zero is the asymptotic lower bound for θ.

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

Pop	Tr	L_{03}	L_m	L_3	C_2	$C_{3,4}$	k_m	INT_{13}	G_j	G_a
Marie	Both	0·779	1·627	2·019	6·590	8·526	6·332	3·088	0·125	0·018
		0·031	0·008	0·013	0·155	0·032	0·346	0·115	0·002	0·001
	High	0·819	1·650	2·050	6·523	8·265	6·332	3·114	0·125	0·019
		0·063	0·012	0·020	0·259	0·544	0·119	0·048	0·003	0·001
	Low	0·738	1·605	1·988	6·657	8·786	6·333	3·063	0·125	0·018
		0·003	0·007	0·012	0·197	0·307	0·103	0·019	0·002	0·001
Klamath	Both	0·743	2·048	2·714	9·553	19·54	7·164	2·975	0·143	0·026
		0·007	0·027	0·042	0·860	1·072	0·088	0·041	0·002	0·001
	High	0·764	2·139	2·862	9·681	20·77	7·211	3·020	0·144	0·026
		0·009	0·023	0·029	0·726	0·813	0·068	0·060	0·001	0·001
	Low	0·722	1·958	2·565	9·425	18·31	7·118	2·930	0·142	0·026
		0·006	0·027	0·042	0·662	2·023	0·172	0·057	0·004	0·001

Standard errors are reported below means.
Pop, population; Tr, treatment; L_{03}, 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); INT_{13}, average interval between clutches (days); G_j, juvenile growth rate; G_a, adult growth rate.

The respective contributions to the vectors $\mathbf{z}_1$ and $\mathbf{z}_2$ of individual phenotypic traits x can be compared by comparing the vector coefficients $z_{x,1}$ and $z_{x,2}$. Resampling can provide confidence intervals for $z_{x,1}$ and $z_{x,2}$.

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 $\mathbf{z}_M$ and $\mathbf{z}_K$, respectively, and the trajectory of divergence between the two populations as $\mathbf{z}_{MK}$. The angle between $\mathbf{z}_M$ and $\mathbf{z}_{MK}$ will be called θ_M, the angle between $\mathbf{z}_K$ and $\mathbf{z}_{MK}$ will be called θ_K, and the angle between the two within-population trajectories $\mathbf{z}_M$ and $\mathbf{z}_K$ will be called θ_{MK} (note that the hypothesis $\theta_{MK}=0$ is analogous to the question 'Is the genetic (co)variance matrix the same in the two populations?' except that we are only concerned with the vector of covariances with size at maturity). 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 $\alpha=0{\cdot}05$, a result is considered significant if $<2{\cdot}5\%$ of bootstrap replicates were smaller (larger) than the observed value).

Broad-sense heritabilities (H^2) and coefficients of genetic variation (CV_G; Houle, 1992) for each trait in each population are reported in Table 2. H^2 was 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 2. *Broad-sense heritabilities (H^2) and coefficients of genetic variation (CV_G) for life-history characters*

	Population			
	Marie		Klamath	
Trait	H^2	CV_G	H^2	CV_G
L_{03}	0·377	0·037****	0·455	0·051****
L_m	0·423	0·079****	0·380	0·077****
L_3	0·655	0·078****	0·489	0·078****
C_2	0·024	0·027	0·199	0·187****
$C_{3,4}$	0·135	0·068****	0·211	0·095****
k_m	0·017	0·012	0·050	0·017*
INT_{13}	0	0	0	0
G_j	0·024	0·026	0·037	0·030
G_a	0·156	0·130****	0	0

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.
**** $P<0{\cdot}0001$; * $0{\cdot}05>P>0{\cdot}01$.

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

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Table 3. *Vector coefficients $z_{x,i}$ of $\mathbf{z}_i$, the trajectory of divergence between two groups*

Trait x	$z_{x,\mathrm{MK}}$	$z_{x,\mathrm{M}}$	$z_{x,\mathrm{K}}$
L_m	**0·166**	**0·297**^{H}	**0·240**^{H}
	(0·135, 0·211)	(0·091, 0·537)	(0·089, 0·528)
L_{03}	0·00026	**0·184**^{H}	**0·161**^{H}
	(−0·015, 0·016)	(0·030, 0·395)	(0·047, 0·372)
L_3	**0·211**	**0·377**^{H}	**0·294**^{H}
	(0·174, 0·263)	(0·101, 0·688)	(0·119, 0·160)
C_2	**0·178**	0·106	0·232^{H}
	(0·019, 0·292)	(−0·547, 0·637)	(−0·742, 0·764)
$C_{3,4}$	**0·939**	−0·034^{H}	0·621^{H}
	(0·911, 0·959)	(−0·975, 0·951)	(−0·597, 0·961)
k_m	**0·087**	0·084	0·049^{H}
	(0·058, 0·123)	(−0·235, 0·416)	(−0·078, 0·258)
INT_{13}	**−0·032**	−0·0518	0·109
	(−0·055, −0·011)	(−0·326, 0·146)	(−0·013, 0·338)
G_j	**0·013**	0·006	0·004
	(0·010, 0·017)	(−0·038, 0·055)	(−0·019, 0·022)
G_a	**0·005**	0·014^{H}	−0·001
	(0·004, 0·007)	(−0·000, 0·035)	(−0·010, 0·007)

Each z_x represents the contribution of phenotypic trait x to the overall divergence between the two groups; $z_{x,\mathrm{MK}}$ is the comparison between the two populations, $z_{x,\mathrm{M}}$ is the comparison between High and Low selected lines in Marie and $z_{x,\mathrm{K}}$ 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.

genetic variation) and Table 3 (vector coefficients z_x). 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 laboratory, but genetic variation for most traits was not much lower in the Marie sample than in the Klamath sample (average CV_G for Marie = 0·051, for Klamath = 0·059), 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 release) 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 error (for example, note that the CV_G of size at birth in Marie is only slightly greater than the CV_G of juvenile 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 genetic variance (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 significantly 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 (θ_{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 irrespective of which population is resampled. With finite data, the results need not be identical because the phenotypic variation within each population will affect the bootstrap distributions of θ_M and θ_K. When θ_M is held constant and compared with the bootstrap distribution of θ_K, $\Pr(\theta_{MK} = 0) > 0·13$; when θ_K is held constant and compared with the bootstrap distribution of θ_M, $\Pr(\theta_{MK} = 0) > 0·38$. Thus, we cannot reject the hypothesis that $\mathbf{z}_M = \mathbf{z}_K$, but the strength of the inference differs depending on which trajectory is arbitrarily held constant.

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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) $z_{x,\mathrm{M}}$ and $z_{x,\mathrm{MK}}$ and (2) $z_{x,\mathrm{K}}$ and $z_{x,\mathrm{MK}}$ (Table 2). Only size at birth (abbreviated L_{03}) 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 $z_{x,\mathrm{M}}$ and $z_{x,\mathrm{K}}$ (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 $\alpha = 0{\cdot}05$. Taking this approach, the level of significance for each z_x is $0{\cdot}05/9$ ($=0{\cdot}0056$), 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 ($z_{\mathrm{L03,K}}$) and the among-population vector coefficient ($z_{\mathrm{L03,KM}}$). For the Marie population, approximately 1·5% of bootstrap replicates of $z_{\mathrm{L03,M}}$ were within the upper 99·5% confidence interval of $z_{\mathrm{L03,KM}}$; 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 – increased body size is associated with increased trait value for other characters, with the sole exception of inter-clutch interval. The trajectory of interpopulation divergence along the axis of body size, clutch size and age at maturity is consistent with the pattern typically observed among species of daphnids. In contrast, within each population, clones selected for extreme divergence in size at maturity diverged significantly only in body size *per se*, whereas clutch size, age at maturity, inter-clutch interval, and juvenile and adult growth rate did not diverge significantly, although the low heritability for some traits precludes a meaningful interpretation of patterns of covariance with those traits (inter-clutch interval, juvenile growth rate). Importantly, however, for no trait did we observe a within-population trajectory that significantly differed in sign from the between-population trajectory (van Noordwijk & de Jong, 1986).

Within both Klamath and Marie, the confidence limits of the vector coefficients of all traits except body size (clutch size, age at maturity, inter-clutch interval, juvenile and adult growth rate) overlap zero. For some traits, particularly clutch size and to a lesser degree age at maturity, the confidence intervals are large and relatively symmetric about zero (Table 3). Biologically, this means that these traits are genetically variable in at least one of the two populations (Table 2) and only weakly correlated with size at maturity. Thus, although we cannot formally reject the hypothesis that the within- and between-population trajectories of divergence are equivalent, there is little evidence for the presence of genetic correlations between body size and other life-history traits. We interpret this result to mean that, on average (see below), alleles that influence body size do not have important pleiotropic effects on other life-history traits. We are therefore led to the conclusion that the multivariate phenotype commonly observed in daphnids may in fact represent the repeated evolution of particularly adaptive suites of traits that are more or less functionally independent of each other.

Offspring size appears to have evolved qualitatively differently from the other traits. In this case, the (lack of) divergence between the two populations differs from that predicted by the genetic architecture within either population. Within both Klamath and Marie, offspring size in the High selected lines was significantly larger than in the Low selected lines (and highly heritable within each population), and yet mean offspring size did not differ significantly between the two populations. This result suggests that stabilizing selection has acted to maintain offspring size within a relatively narrow range relative to the diversification of other traits observed in these populations. This result is not inconsistent with the conclusion that multivariate phenotypic evolution has been unhindered by pleiotropic constraints, and in fact reflects 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

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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 ($z_{x,KM}$) 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 with heritabilities 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 offspring 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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