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The evolution of *Daphnia pulex* in a temporally varying environment

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Summary

We investigated three aspects of adaptation to variable environments in *Daphnia pulex* (Cladocera: Crustacea): (1) effects of temporal variation on the evolution of phenotypic plasticity; (2) plasticity in sexual versus asexual lineages; (3) maintenance of genetic variation in variable environments. We performed a 72-day quasi-natural selection experiment comparing three patterns of variation: constant temperatures, varying but predictable temperature change, and unpredictable temperature change. All populations were begun with an identical array of 34 clones. During selection clonal variation declined in all populations and different patterns of environmental variation had little effect on amounts of genetic variation. Sexual and asexual lineages differed in size and growth rate, but did not differ in amounts of plasticity or in adaptation to variable environments. The primary target of selection was the Malthusian parameter (r) and life history traits of development time, offspring size and offspring number. The heritability of plasticity was generally lower than trait heritability. Because of this difference, the selection response on the mean of the traits overwhelmed the selection response on plasticity. Lower heritabilities of plasticity are very typical, suggesting that our results will be typical of responses to selection in nature. Our results suggest that selection will act mostly on trait means within environments and that plasticity will evolve often as a correlated trait. Because selection on plasticity is based on its across-deme, global fitness, this process will usually be slow. Comparative studies need to shift from closely related, local population differences to those of more distantly related populations or even different species.

1. Introduction

(i) *Primary question – phenotypic plasticity*

Nearly all organisms live in environments that change through time. If these changes are such that different phenotypes have maximal fitnesses at different times, then this environmental variation can lead to one of three evolutionary outcomes: (1) individuals can be phenotypically plastic, changing to match the optimal phenotype at each time; (2) individuals can be phenotypically fixed and specialized for different optimal phenotypes; (3) individuals can be phenotypically fixed and generalized, never having the optimal phenotype at any one time but having the greatest average fitness across all times. Which of these outcomes, or mixture of outcomes, occurs depends on a variety of factors. In this paper we explore two: (1) how patterns of environmental variation affect the alternative evolutionary responses; (2) how trait and plasticity heritabilities affect those responses. Here we are primarily concerned with factors favouring plasticity and so compare plastic individuals with fixed-phenotype individuals, lumping categories two and three above.

The conditions that favour each of the outcomes are addressed by a number of theoretical models (Scheiner, 1993*a*). These models, while based on a variety of different approaches and assumptions, agree on the conditions favouring each type of individual. Which outcome is favoured depends on two factors: the rate at which the environment changes relative to the response rate of the organism and the predictability of any changes. If the organism can respond im-

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mediately to environmental changes (e.g. physiological responses), then plasticity is always favoured. In contrast, if the organism's phenotype becomes fixed at a single developmental point (e.g. adult size in holometabolous insects), then plasticity is favoured only if the environmental cue at the determination point accurately predicts the future environment. That is, plasticity is favoured when the environment is variable but predictable. If the environment is variable but unpredictable (the environment changes randomly between the time of development of the trait and the time of selection), then the population will evolve to a single phenotype that represents the optimal compromise among environmental states. A theoretical treatment of traits which are changeable within the lifetime of the individual but at a rate much slower than that of the environment has not been formally done. Scheiner (1993*a*) postulated that plasticity would be favoured only when environmental states are strongly correlated, similar to fixed traits.

Despite the general agreement of theoretical models, few data exist testing the above predictions. As predicted by theory, labile traits of physiology and behaviour generally are plastic in an adaptive manner (Scheiner, 1993*a*). Studies of fixed traits in natural populations are more equivocal, supporting the prediction that variable environments will select for plasticity in some traits and some species (Cook & Johnson, 1968; Garbutt & Bazzaz, 1987; Etges, 1989; Rabinowitz *et al*., 1989; Fox, 1990; Semlitsch *et al*., 1990). However, these data were not collected to directly test these predictions. In particular, a true test requires that exactly the same environmental factor be measured in the field and manipulated in the laboratory when measuring plasticity. More direct tests of these predictions are needed. In this paper we take an alternative approach to test these predictions through the use of a laboratory selection experiment.

Surrounding this entire issue is the more general question of selection on and the evolution of phenotypic plasticity. How often is plasticity the direct object of selection as opposed to evolving as a correlated response to selection on trait means? This question has been contentious with alternating claims (Scheiner, 1993*b*; Schlichting & Pigliucci, 1993; Via, 1993; Via *et al*., 1995). Recent theoretical work on the evolution of plasticity in a spatially variable environment shows that plasticity evolves as a function of its global fitness across all environments (Zhivotovsky *et al*., 1996; Scheiner, 1998). This evolution can be opposed by local (within-deme) selection on trait means. Similarly, in a temporally varying environment, the evolution of plasticity depends on the long-term fitness of a plastic genotype, and might be opposed by short-term evolutionary responses. Complicating this picture are likely differences between the heritability of plasticity across environments and the heritability of traits within a single environment. In general, trait plasticities have lower heritabilities than those of the traits themselves (Scheiner, 1993*a*). So, all other things being equal, the evolutionary response of trait plasticity is expected to be less than predicted by an optimality analysis.

(ii) *Two other questions*

Because of the way we crafted our experiments, we were able to address two additional issues involving the maintenance of sex and genetic variation. Although these were not the primary motivation for this study, we would be remiss if we ignored them. They provide a lagniappe to the core set of experimental results. In particular we ask: (1) whether different modes of reproduction (sexual *v*. asexual) are differentially adapted to different patterns of environmental variation; (2) whether different amounts and patterns of environmental variation maintain different amounts of genetic variation.

The maintenance of sex – genetic recombination – remains a central issue in evolutionary biology (Michod & Levin, 1988). Hypotheses to explain the maintenance of meiosis and sexual reproduction fall into three categories: (1) the environmental change hypotheses posit a greater fitness for lineages that produce variable progeny in a variable environment; (2) the tangled bank hypothesis posits a greater fitness for lineages that reduce competition among siblings by having those individuals be phenotypically variable; (3) the Red Queen hypothesis posits a greater fitness for lineages that can continually evolve to escape predators or parasites. However, sex is not the only way to produce variable progeny. They can also be produced by two developmental mechanisms: phenotypic plasticity and developmental variability. No formal analysis has been done to examine evolutionary outcomes when plasticity and developmental variability are alternatives to recombination. However, Scheiner (1993*a*) postulated that in variable environments plastic, asexual lineages would be favoured over lineages that were non-plastic, genetically variable and sexual, because cues to ensure the development of variable phenotypes will always be present. From this conjecture, we predict that in a variable environment asexual lineages would be more plastic than sexual lineages as the latter can be maintained by a combination of plasticity and specialization. The proof of our conjectures would suggest what ecological conditions favour asexual or sexual lineages. Theory indicates that asexual reproduction is favoured in either stable environments (Eshel & Feldman, 1970; Maynard Smith, 1980) or frequently fluctuating ones (Charlesworth, 1976; for review see Kondrashov, 1993). Empirical evidence shows, however, that asexual reproduction is more

frequently observed in novel and disturbed environments (Bell, 1982). Sexual and asexual lineages have not, until now, been compared for levels of trait plasticity.

The maintenance of genetic variation has been of longstanding interest (Hedrick, 1986). Considerable effort has been expended on the development of models explaining how environmental variation can result in such maintenance. In brief, the conditions under which temporal variation will maintain genetic are very stringent, while spatial variation is more likely to maintain variation. A number of experiments using various species of *Drosophila* (Powell, 1971; McDonald & Ayala, 1974; Minawa & Birley, 1978; Powell & Wistrand, 1978; Oakeshott, 1979; Mackay, 1980; Haley & Birley, 1983) and *Chlarmdomonas* (Bell, 1997; Bell & Reboud, 1997; Reboud & Bell, 1997) have addressed this issue (see review in Hedrick, 1986). Those experiments found that temporal or spatial environmental variation tended to retard the loss of genetic variation compared with constant environments. The magnitude of the effects varied among experiments, however, with the smallest effects found in temporally varying environments. Our experiment provides another test of these conclusions with a different experimental animal and a comparison of two different patterns of temporal variation.

(iii) *The experiments in brief*

In this paper we address these three issues with two experiments using as a model system the freshwater crustacean *Daphnia pulex* (Cladocera: Crustacea) and its plasticity in response to temperature variation. We chose *Daphnia pulex* because this species has both sexual and asexual lineages and previously was used extensively in studies of plasticity (e.g. Lynch *et al*., 1989; Spitze *et al*., 1991; Spitze, 1992). Our first experiment consisted of quasi-natural selection (Scheiner, 1999). This experiment was designed to address all three issues simultaneously: the evolution of plasticity, the differential adaptation of sexual and asexual lineages to environmental variation, and the maintenance of genetic variation. We compared the evolution of populations in three contrasting types of environments: (1) constant; (2) variable and unpredictable; (3) variable but predictable. Selection favouring plasticity requires two conditions: environmental variation and predictable cues (Gavrilets & Scheiner, 1993). Thus, the expected outcome was that the first two types of environment (Constant and Unpredictable) would favour genotypes with a fixed phenotype while the third type of environment (Predictable) would favour genotypes that were phenotypically plastic. The primary target of selection was the Malthusian parameter (r) and, through that, life history traits of development time, offspring size, and offspring number. The populations consisted of a mixture of sexual and asexual lineages to test wether past natural selection has differentially adapted them to variable environments. If asexual lineages are more plastic, then they would be favoured in the Predictable environment. Because we tracked the genetic composition of the populations during the experiment, we could observe whether different amounts and patterns of environmental variation were more conducive to the maintenance of genetic variation.

Our second experiment examined the plasticity of morphological and life history differences among these lineages. These results are used both to interpret the outcome of the selection experiment and to compare past evolution of the sexual and asexual lineages. Thus, our experiments had three goals: (1) testing predictions of plasticity evolution under different types of temporal variation; (2) comparing sexual and asexual lineages for amounts of plasticity and adaptation to variable environments; (3) examining effects of different amounts and patterns of environmental variation on the maintenance of genetic variation.

2. Materials and methods

(i) *Study organism*

Daphnia pulex is a small (*c*. 1–2 mm) freshwater filter-feeder. Reproduction occurs by one of two modes: direct developing young or diapausing eggs. Direct developing individuals are always produced parthenogenetically, one brood during each adult moult. The cycle is as follows. Shortly after the adult moults she releases ova into her ovaries. After completing initial development, the eggs are deposited in the brood chamber on the back of the adult following her next moult. The eggs then develop into juveniles in the brood chamber and are released during the subsequent moult. Thus, at any one time the adult has offspring at three stages of development: unreleased ova, developing eggs in the ovaries, and developing juveniles in the brood chamber. Following release, the juveniles go through three to five instars, depending on conditions, before becoming adults. The first adult instar is defined as the instar in which the first brood is deposited in the brood chamber. Adults may live up to 30 instars, but typically no more than four to six instars in the field. Diapausing eggs differ in several ways. First, the clutch size is always two. Secondly, the eggs are larger and differ in their relative proportions of proteins and lipids. Thirdly, the eggs can be produced either parthenogenetically or sexually depending on the genotype of the clone, defining two reproductive types. These two types are usually referred to as asexual and cyclically parthenogenetic, respectively. For simplicity and clarity we will refer to

them as asexual and sexual, with the reminder that sexual genotypes still produce all direct developing offspring by parthenogenesis.

The clones used in our experiment came from populations in northern Illinois and Indiana. Sixteen of the clones were from eight strictly asexual populations. The asexual status of the populations was determined by: (1) the lack of Hardy–Weinberg frequencies in the populations; (2) the low number of genotypes in the populations (Hebert *et al.*, 1988); (3) the lack of males. The remaining 18 clones were from a single cyclically parthenogenetic population, PA of Lynch *et al.* (1989); our sample had allozyme genotypes matching those previously reported for this population. From these populations we chose clones that were electrophoretically unique based on the following loci: *Pgi*, *Pgm*, *Got*, *Est*, *Alk*. Clones were maintained in the laboratory in 200 ml of distilled water with chemicals added to mimic pond water (Lynch *et al.*, 1986) and fed excess amounts of *Scenedesmus acutus* from cultures in exponential growth. Densities of the stock cultures were not controlled.

Selection: experiment one

Our first experiment consisted of quasi-natural selection carried out for 72 d, or approximately 10 parthenogenetic generations. This experiment met the goals of: (1) testing predictions of the evolution of plasticity under different types of temporal variation; (2) determining differential adaptation of sexual and asexual lineages to environmental variation; (3) examining patterns of the maintenance of genetic variation. The experiment consisted of three treatments, two replicate populations per treatment, with one of the following temperature patterns: (1) The temperature was held at a constant 20 °C (Constant); (2) the temperature was randomly switched between 17 °C, 20 °C; 23 °C every 3 d with the constraint that the total time spent at each temperature was equal across the entire experiment (Unpredictable). That is, there were 24 3 d blocks at each temperature that were distributed in a random order; (3) the temperature was varied in a regular fashion consisting of 12 d at 17 °C, 6 d at 20 °C, 12 d at 23 °C, 6 d at 20 °C, then the entire cycle was repeated once more (Predictable). Again, the total time spent at each temperature was equal across the entire experiment.

These patterns and amounts of temperature variation are comparable to those of small ponds in this region. Thus, these treatments mimic typical patterns of variation and would probably have been faced by these clones previously. Under these conditions time to maturity was 4–6 d and the interclutch interval was 2 d. This species can alter clutch size and offspring provisioning within 2 d of being placed at a new temperature (Yampolsky & Scheiner, unpublished data). Thus, the time scale of change for the third treatment is predictable for these organisms with respect to reproductive traits.

Each replicate consisted of a tank containing 18 l of artificial pond water. Food was supplied daily at a rate of 0·5 μgC/ml (5×10^4 cells/ml) of *S. acutus*. Cell density was determined with a spectrophotometer and supplied from a single daily mass sample to ensure uniformity across treatments. Daylength was set at 14 h light/10 h dark with light supplied by 'daylight' fluorescent tubes.

Initially, each replicate consisted of six individuals (two adults and four juveniles) of each of the 34 clones. Thus, each replicate began with exactly the same genetic composition. Populations were allowed to grow naturally by asexual (direct developing) reproduction. Every 6 d each tank was drained, the *Daphnia* were filtered out, and a random sample of 500 individuals used to re-establish the population. The population bottleneck size was chosen to minimize the chance of loss of clones through drift. This procedure kept the population in a state of continual growth. Population densities were estimated every 3 d by means of a vertical tow. Clonal frequencies were measured every 18 d using a sample of 41–117 individuals per population taken from the population excess. During this time we concluded that three pairs of asexual clones could not be reliably distinguished, so they were lumped for purposes of determining frequencies. Changes in frequencies were assessed both as number of clones and as Shannon–Weiner diversity, which takes into account both the number of clones and their relative frequencies. It equals the number of clones if frequencies are equal and approaches one as a single clone comes to dominate the population.

(ii) *Phenotypic assessment: experiment two*

The second experiment consisted of assessing the phenotype of each clone. This experiment met the goals of: (1) testing plasticity evolution; (2) comparing sexual and asexual lineages for amounts of plasticity. Individuals were raised and measured using the following procedure. We took from each clone in the stock cultures a single adult individual. Eight offspring of this individual, usually from a single clutch, were placed individually into vials with *c.* 25 ml of water, four each at 17 °C and 23 °C. Food levels and lighting conditions were the same as during the selection experiment. These individuals were measured for the following traits: length at birth, number of hours to maturity, length at maturity and, for the first clutch, length of mother, clutch size, and the length at birth of three offspring. Maturation time was determined to the nearest 6 h by monitoring individuals twice daily

and correcting moult time using the development stage of the individuals in the brood chamber (Lynch *et al*., 1989). Length measurements were done with a LASICO ocular filar on a Wild stereomicroscope and an S-4A Auto-processor. Lengths were converted to biomass using the following, empirically derived, relationships:

$$17\ ^\circ\mathrm{C}: \log_{10}(\text{mass})\,(\mu\text{g}) = 1{\cdot}073\,(\pm 0{\cdot}065) + 1{\cdot}684\,(\pm 0{\cdot}248)\log_{10}(\text{length})(\text{ml}) \quad (n = 30, r^2 = 0{\cdot}61),$$

$$23\ ^\circ\mathrm{C}: \log_{10}(\text{mass})\,(\mu\text{g}) = 0{\cdot}788\,(\pm 0{\cdot}124) + 2{\cdot}481\,(\pm 0{\cdot}484)\log_{10}(\text{length})\ (\text{ml}) \quad (n = 39, r^2 = 0{\cdot}40).$$

Values in parentheses are 1 SE. Mass gain of the mother during the first adult instar was determined as the difference in estimated masses based on her length following release of the first clutch into the brood pouch and her length for the previous instar. Clutch mass was estimated using the mean offspring length, converted to mass, times the clutch size. Reproductive effort was calculated as the clutch mass divided by clutch mass plus growth mass increment. The intrinsic rate of increase (r) was calculated as ln(clutch size)/(time to first clutch). This measure of r is somewhat inaccurate because it only accounts for the first clutch. However, measures of r using more clutches are typically highly correlated (Spitze *et al*., 1991). We could only measure the first clutch because during the experiment contamination of the water supply killed the experimental animals. One result of these deaths is that, of the original 34 clones, we were unable to measure four, while four others were measured in just the 23 °C environment. For the key trait r we lacked estimates for eight clones. Thus, we probably underestimated genetic variation and estimates of the means may be biased. In particular, the slowest developing clones were most likely to be missing from our estimates.

These measures were used to determine differences in the phenotypes of the sexual and asexual clones. Trait differences of the two reproductive types were determined using SAS procedure GLM (SAS Institute, 1989) and the model

$$\sigma^2_{\mathrm{P}} = \sigma^2_{\mathrm{E}} + \sigma^2_{\mathrm{R}} + \sigma^2_{\mathrm{R\times E}} + \sigma^2_{\mathrm{G(R)}} + \sigma^2_{\mathrm{G(R)\times E}} + \sigma^2_{\mathrm{e}},$$

where σ^2_{P} is the total phenotypic variation across treatments and clones, σ^2_{E} is the across-treatment (environmental) variation, σ^2_{R} is the between-reproductive type variation, $\sigma^2_{\mathrm{R\times E}}$ is the reproductive type–environment interaction variation, $\sigma^2_{\mathrm{G(R)}}$ is the within-reproductive-type among-clone (genotypic) variation, $\sigma^2_{\mathrm{G(R)\times E}}$ is the genotype–environment interaction variance, and σ^2_{e} is the residual variance. Environments and reproductive types were treated as fixed effects and clones (genotypes) within types were treated as random effects. Type III sums-of-squares were estimated; *F*-tests were done using the 'Test' option, which computes a Satterthwaite test correcting for an unbalanced design. Heritabilities within each environment were calculated as $\sigma^2_{\mathrm{G}}/\sigma^2_{\mathrm{P}}$ with variance components estimated within each environment. Variance components were estimated using procedure VARCOMP and the restricted maximum likelihood option, which constrains estimates to be non-negative.

The phenotypes of the populations at the end of selection were estimated by using trait means of each clone estimated during the phenotypic assessment weighted by the frequency of each genotype at the end of the selection experiment. Plasticity was calculated as the difference in clonal means (value in 17 °C minus value in 23 °C), again weighted by clone frequency. Differences in population phenotypes were tested by use of a two-way ANOVA with temperature and population as fixed effects. A significant population effect indicates that the populations diverged during selection. A significant population–temperature interaction effect indicates that the populations diverged with respect to trait plasticity. The mortality during the phenotypic assessment had a lesser effect on these comparisons as only two of the unmeasured clones existed in substantial frequencies in any of the final populations. That is, the slowest developing clones which were not measured in the phenotypic assessment were also the ones most frequently eliminated during the selection experiment.

3. Results

(i) *Selection: experiment one, goals one and three*

During the course of the selection experiment, over the 6 d period between water changes, total population densities varied between 30 and 200 individuals/l – 600 and 4000 individuals per population – and adult densities varied between 15 and 20 individuals/l (Fig. 1). Clonal variation declined quite rapidly during the first 18 d, then more slowly thereafter in most populations (Fig. 2). During the first 5 weeks Shannon–Weiner diversity dropped by *c*. 50% and the number of genotypes declined by one-third. Only three genotypes were lost in all six populations. While populations differed significantly in final clonal diversities, there was no relationship with the type of environmental variation; the final ordering was P2 > U2 > P1 > C1 > C2 > U1. For example, population U1 had the lowest diversity while population U2 had the second highest; this difference was statistically significant ($Z = 5{\cdot}14$, $P < 0{\cdot}0001$). While both Predictable replicates had higher final diversities than both Constant replicates, P1 did not differ significantly from C1 ($Z = 0{\cdot}26$, $P < 0{\cdot}8$).

Population divergence at the end of the 72 d experiment was assessed using multidimensional

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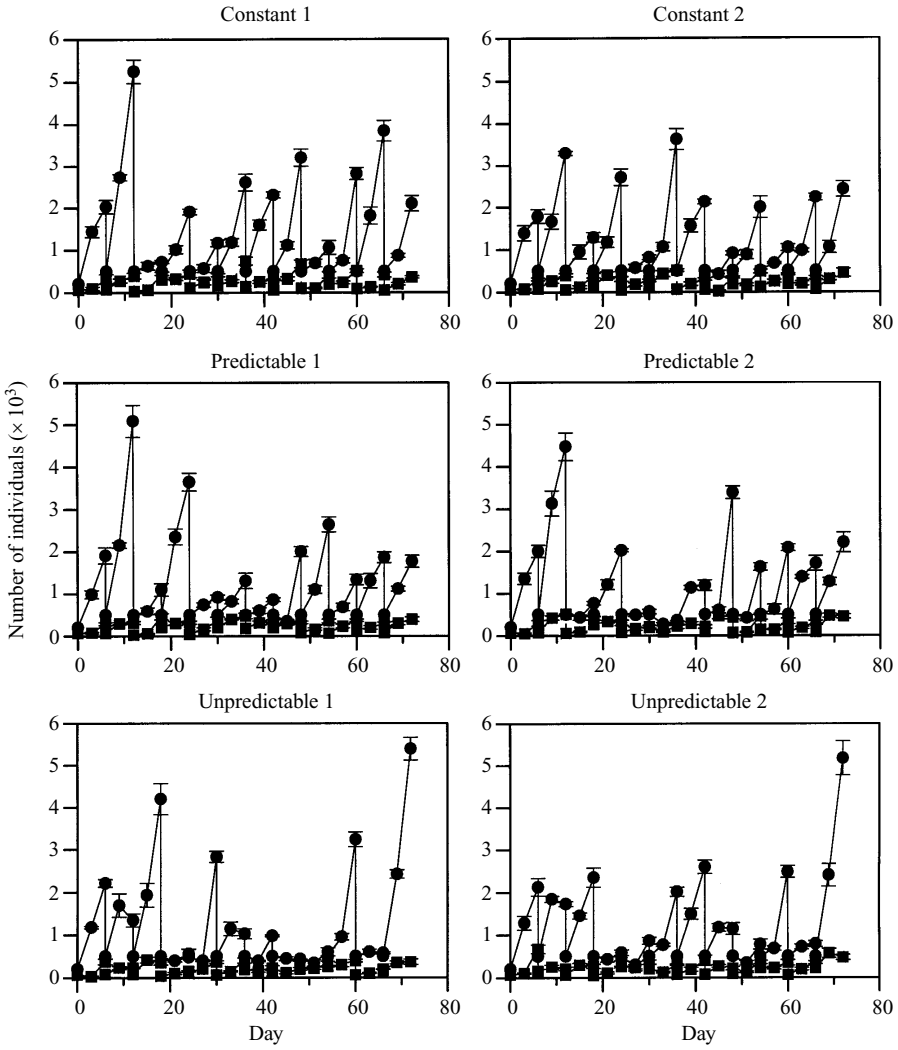

Fig. 1. Population densities (mean ± 1 SE) over the 72 d of the quasi-natural selection experiment of each of the six lines showing the total number of individuals (circles) and the number of adults (squares).

scaling (Fig. 3). All populations diverged from the base population – an expected result given the large decrease in genetic variability. The most divergent responses occurred in the Unpredictable treatment, with the two replicates differing substantially both from the other treatments and from each other. The two replicates of the Constant and Predictable treatment tended to cluster.

The amount of genetic change was much greater than expected by drift alone. The populations went through 11 bottlenecks consisting of 500 individuals each. The probability of losing one clone through drift alone through all of those bottlenecks is less than 0·012%. Given the large observed losses (Fig. 2), changes in these populations were most likely due to selection.

(ii) *Phenotypic assessment: experiment two, goal one*

Rearing temperature affected time to maturity, adult growth, and clutch mass (Table 1). That is, these traits were plastic. Traits that were not plastic under these conditions included length at birth, length at maturity, clutch size, and reproductive effort. No statistically significant interactions were found between temperature and clones (genotypes) except for a weak effect on reproductive effort. These populations lacked (or had very low levels of) genetic variation for plasticity. Of particular interest is the heritability of r as it was the direct object of selection. The heritability of r was 0·16 and 0·11 at 17 °C and 23 °C, respectively. In contrast, the heritability of plasticity of r was zero; no genotype–environment variation was found for this trait (Table 1).

The final populations differed, on average, for all traits (Table 2). They differed with respect to plasticity – significant population–temperature interaction – for all but one trait, the intrinsic rate of increase (Table 3). The differences did not follow those predicted by theory. Population C2 was the most plastic for seven of the traits while P2 was the least plastic for two – exactly the opposite of the predictions. For r, the

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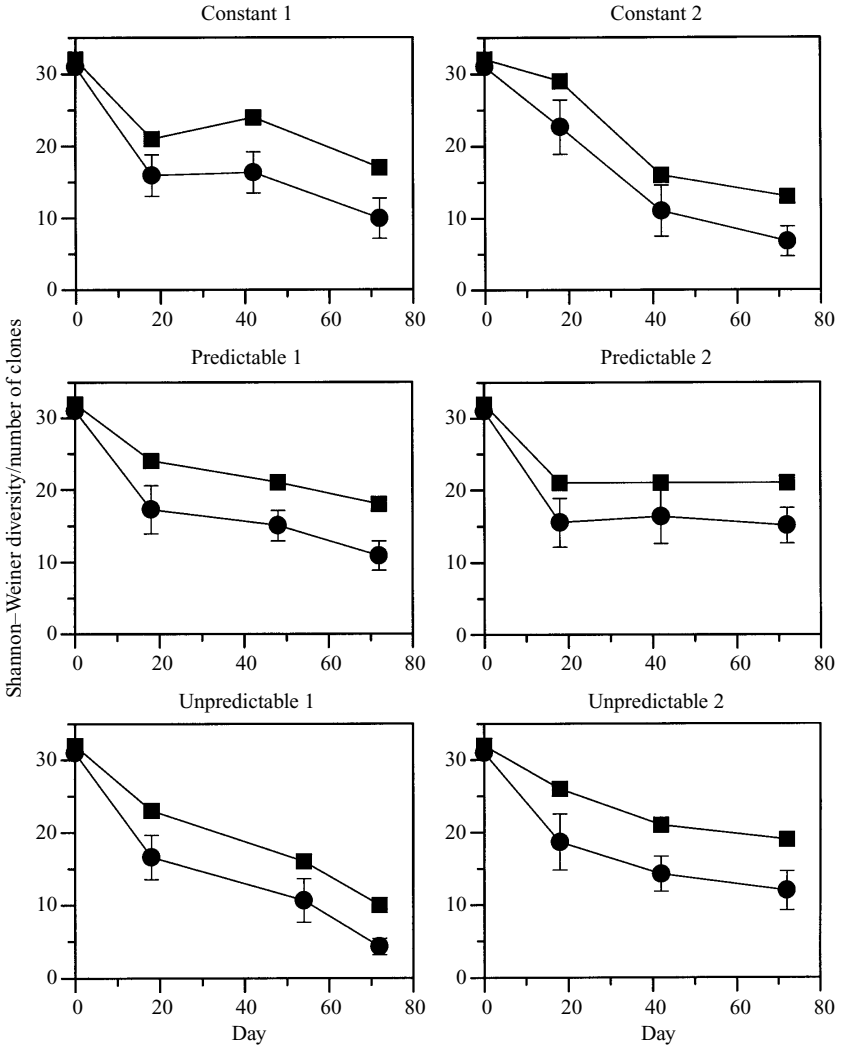

Fig. 2. Changes in clonal variation over the 72 d of the quasi-natural selection experiment of each of the six lines as measured by the exponent of Shannon–Weiner diversity (circles) or number of clones found (squares). The two values are equal when clones are equally frequent. Bars show 95% CI.

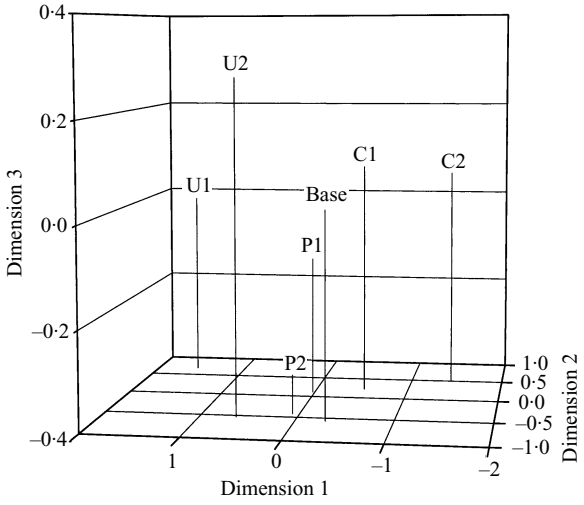

Fig. 3. Clonal divergence of the six lines from the starting frequencies after 72 d of quasi-natural selection measured as Euclidean distance of clone frequencies and assessed using the multidimensional scaling procedure of SYSTAT (Wilkinson, 1987).

direct object of selection, while population P2 was the most plastic, population P1 was among the least plastic. In general, there was little consistency between the replicates for a given treatment. In the analysis of the final populations, all traits were plastic (Table 2). That is, over all populations and traits, selection favoured more plastic genotypes as indicated by the increased plasticity following selection (compare *Temperature* in Tables 1 and 2). The difference compared with the analysis of the base population (Table 1) gave more power to the second analysis to the detection of differences in *Temperature* because of the use of a different statistical model (testing against *Error* rather than *Temperature* × *Clone*) and the weighing of clones by their final frequencies.

(iii) *Reproductive type: experiments one and two, goal two*

The two reproductive types (sexual and asexual) were eliminated with equal frequency among the selection

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Table 1. *ANOVA* (*MSs*) *of morphological and life history traits of sexual and asexual clones of* D. pulex

Trait	Covariate	Temperature, T	Reproductive type, R	T × R	Clone, C (*R*)	T × C (*R*)	Error	*n*
Length at birth	**0·0194**[a]	0·0010	**0·0257**	0·0008	0·0016	0·0008	0·0008	113
Length at maturity	0·0331[b]	0·0061	**0·9047**	0·0386	**0·0473**	0·0189	0·0197	173
Mass gain at first adult instar	**1053·2**[c]	**537·4**	**183·7**	1·1	7·9	4·5	9·3	154
Time to maturity	–	**7·6260**	0·0441	0·0003	**0·0385**	0·0192	0·0161	175
Size of first clutch	5·24[a]	1·85	2·69	3·21	**6·93**	2·74	4·30	142
Mass of first clutch	**392·1**[c]	**2074·6**	8·2	137·1	75·1	72·7	85·9	124
Reproductive effort	–	0·092	0·210	0·003	0·074	**0·117**	0·067	129
Intrinsic rate of increase	–	**0·1237**	0·0000	0·0001	**0·0118**	0·0043	0·0055	149

Significant ($P < 0·05$) effects are shown in bold. Differences in sample sizes among traits are due to mortality during the experiment (see text) or lost data.
[a] Length of mother in previous instar.
[b] Length at birth.
[c] Mass of mother in previous instar.

Table 2. *ANOVA* (*MSs*) *of morphological and life history traits of the six populations of* D. pulex *at the end of 72 d of quasi-natural selection*

Trait	Covariate	Temperature, T	Population, P	T × P	Error	*n*
Length at birth	**0·7646**[a]	**0·0277**	**0·0041**	**0·0022**	0·0007	788
Length at maturity	**4·594**[b]	**1·962**	**0·117**	**0·037**	0·011	884
Mass gain at first adult instar	**356·0**[c]	**692·2**	**18·7**	**18·2**	3·1	884
Time to maturity	–	**743710**	**670**	**1036**	151	884
Size of first clutch	**151·3**[a]	**120·0**	**9·1**	**5·5**	1·4	846
Mass of first clutch	**9285**[c]	**11092**	**192**	**549**	23	796
Reproductive effort	–	**0·5826**	**0·0936**	**0·0998**	0·0179	796
Intrinsic rate of increase	–	**0·3877**	**0·0157**	0·0019	0·0022	846

Significant ($P < 0·05$) effects are shown in bold. Time to maturity was log-transformed prior to analysis.
[a] Length of mother in previous instar.
[b] Length at birth.
[c] Mass of mother in previous instar.

treatments (Fig. 4). Of the six populations, three (C1, P1, U2) did not differ in the final proportion of asexual clones either from each other or from the original proportion. Of the other three, populations P2 and U1 had a significantly greater proportion of sexual clones while population C2 had a significantly greater proportion of asexual clones. Thus, while replicates differed in their final proportions of asexual clones, treatments did not differ.

We next compared the reproductive types for morphological and life history traits. Asexuals were larger than sexuals as measured by length at birth, length at maturity, and mass gain during the first adult instar (Table 4). However, the two reproductive types did not differ with respect to traits determining reproductive rate: time to maturity, clutch size, reproductive effort, and the intrinsic rate of increase. Thus, morphological differences did not result in life history differences. This lack of differences was not simply due to a lack of genetic variation for these traits. Genetic variation among clones was found for both clutch size and the intrinsic rate of increase (Table 1). No statistically significant interactions were found between temperature and reproductive types. That is, sexual and asexual clones were equally plastic.

4. Discussion

We failed to confirm two of the theoretical propositions: (1) Plastic genotypes were not preferentially selected for in the variable but predictable environment; (2) sexual *v*. asexual reproductive types were neither different in their levels of plasticity nor different in their adaptation to various patterns of environmental heterogeneity. On the other hand, we did confirm the prediction that temporal variation is unlikely to maintain genetic variation. Negative results are always difficult to interpret. They may indicate

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Table 3. *Means for morphological and life history traits of the six populations of* D. pulex *at the end of 72 d of quasi-natural selection*

Trait	Temperature	Population C1	C2	P1	P2	U1	U2
Length at birth (mm)	17	0·621	0·642	0·625	0·616	0·574	0·626
	23	0·607	0·620	0·616	0·610	0·572	0·615
	Plasticity	$0·0142^{A}$	$0·0210^{A}$	$0·0097^{AB}$	$-0·0005^{B}$	$0·0035^{B}$	$0·0096^{AB}$
Length at maturity (mm)	17	1·71	1·75	1·73	1·66	1·64	1·75
	23	1·63	1·65	1·66	1·64	1·54	1·65
	Plasticity	$0·072^{A}$	$0·077^{A}$	$0·075^{A}$	$0·014^{B}$	$0·099^{A}$	$0·080^{A}$
Mass gain at first adult instar (μg)	17	7·42	7·66	7·64	7·09	7·48	7·97
	23	8·94	9·87	9·54	8·88	9·52	8·83
	Plasticity	$-1·20^{BC}$	$-2·29^{A}$	$-1·26^{BC}$	$-1·60^{AB}$	$-2·01^{AB}$	$-0·53^{C}$
Time to maturity (h)	17	173·9	170·5	168·0	170·5	167·7	168·5
	23	107·9	101·8	108·6	110·7	116·2	110·7
	Plasticity	$64·0^{AB}$	$66·5^{A}$	$56·7^{CD}$	$58·2^{BC}$	$51·3^{D}$	$55·2^{CD}$
Size of first clutch	17	5·6	6·0	5·5	5·3	4·5	5·2
	23	4·6	4·2	4·4	4·8	3·8	4·4
	Plasticity	$1·03^{BC}$	$1·79^{A}$	$1·19^{B}$	$0·54^{C}$	$0·70^{BC}$	$0·86^{BC}$
Mass of first clutch (μg)	17	30·52	34·79	29·89	28·31	21·14	28·66
	23	8·70	8·23	8·76	8·88	6·06	8·22
	Plasticity	$22·11^{B}$	$26·94^{A}$	$21·62^{B}$	$20·03^{B}$	$15·20^{C}$	$20·33^{B}$
Reproductive effort (%)	17	78·0	79·5	74·8	71·9	70·2	71·2
	23	71·2	68·8	67·8	68·6	74·3	62·9
	Plasticity	$7·5^{A}$	$12·0^{A}$	$8·5^{A}$	$4·8^{A}$	$-3·6^{B}$	$10·0^{A}$
Intrinsic rate of increase (r) (d^{-1})	17	0·167	0·177	0·163	0·165	0·153	0·206
	23	0·215	0·206	0·208	0·215	0·186	0·200
	Plasticity	−0·051	−0·031	−0·044	−0·057	−0·044	−0·050

Differences in plasticities were determined by a Tukey's test; different superscripts denote significant differences ($\alpha = 0·05$).

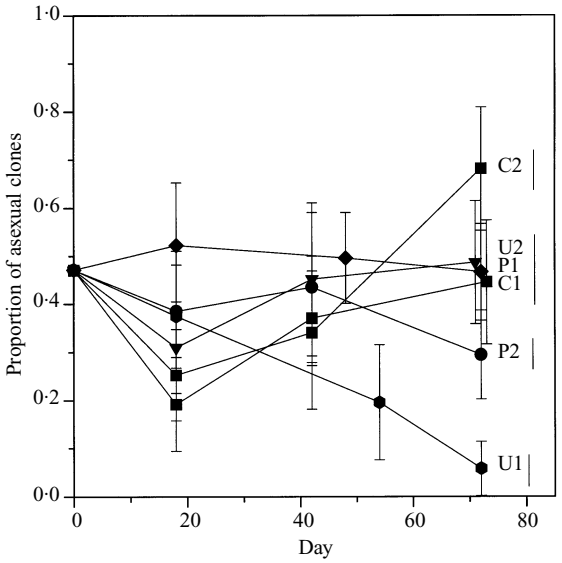

Fig. 4. Changes in the proportion (±95% CI) of asexual clones in each of the six lines over the 72 d of the quasi-natural selection experiment. Lines connect populations that do not differ significantly ($P < 0·05$) at day 72 based on a Tukey's test of multiple proportions (Zar, 1996, p. 561). Symbols are offset at day 72 for clarity.

that the theory is incorrect, that one or more theoretical assumptions were not met, or that a flaw existed in the execution of the experiment. Statistical power, while sometimes a factor in negative results, is not at issue here. We had more than sufficient power to measure differences among populations (Table 2) and between reproductive types (Table 4). While the number of replicates was low, the complete lack of agreement with theoretical predictions suggests that more replicates would not have altered the conclusions. Similarly, differences between sexual and asexual lineages were so small (Table 4) that more clones would not have changed the conclusions. As we discuss below, these negative results do illuminate aspects of the evolution of phenotypic plasticity.

(i) *Plasticity and patterns of environmental variation: goal one*

First, we consider the prediction that plasticity should be favoured in variable but predictable environments. While evolution during the experiment created differences in plasticities among the populations, those differences did not conform to theoretical expectations. In particular, the populations did not differentiate with respect to the plasticity of r. The failure of our selection experiment to meet the theoretical prediction has a straightforward explanation. Selection was on r, the Malthusian parameter. The heritability of the plasticity of r was substantially

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Table 4. *Means (95% CI) for morphological and life history traits of sexual and asexual clones of* D. pulex

Trait	Reproductive type		Test of differences	
	Sexual	Asexual	*F*	*P*
Length at birth (mm)	0·583 (0·572, 0·593)	0·647 (0·637, 0·658)	21·11	0·0001
Length at maturity (mm)	1·57 (1·51, 1·62)	1·75 (1·70, 1·81)	25·21	0·0001
Mass gain at first adult instar (μg)	6·84 (6·25, 7·43)	7·77 (7·18, 8·36)	21·55	0·0001
Time to maturity (h)	139·8 (130·8, 148·8)	138·7 (129·7, 147·6)	1·31	0·3
Size of first clutch	4·8 (4·2, 5·5)	5·0 (4·3, 5·6)	0·47	0·5
Mass of first clutch (μg)	13·31 (11·26, 15·37)	20·06 (18·01, 22·12)	0·10	0·8
Reproductive effort (%)	69·0 (50·5, 76·8)	68·6 (51·5, 64·8)	2·95	0·1
Intrinsic rate of increase (r)	0·192 (0·170, 0·215)	0·192 (0·174, 0·211)	0·0003	0·99

Significant differences between reproductive types were determined by ANOVA (Table 1).

less than the heritability of r itself. Therefore, the response to selection on r overwhelmed any selection on the plasticity of r or any of its life history components. The theoretical prediction might have been met if either selection was able to proceed for a long enough period of time or the initial population had more genetic variation for plasticity.

One might expect selection to favour equally high values of r in all environments. In that case the lack of differences in the plasticity of r is expected. However, this lack of plasticity in fitness itself must be maintained by plasticity in other traits. For example, for exotherms fewer large offspring are favoured at low temperatures and more small offspring are favoured at high temperatures (Yampolsky & Scheiner, 1996). So we expected that clutch size and length at birth would respond to differences in environmental variation. Yet, for these traits at the end of selection, population C2 was the most plastic while population P2 was the least plastic (Table 3). Thus, our conclusions are not simply an artefact of low levels of genetic variation for r but extend generally to other traits.

Our results have implications for the evolution of plasticity in natural populations. Trait plasticities almost always have lower amounts of genetic variation than the traits themselves (Scheiner, 1993*a*). Thus, natural populations will often respond to selection in ways similar to this experiment. Selection on plasticity will often be secondary to selection on trait means. For temporally varying environments, a generalist strategy is more likely than a plastic strategy (Van Tienderen, 1997). Currently there are no data from natural populations to confirm this conjecture. Doing so requires a comparison of populations from the three types of environments: constant, variable but predictable, and variable but unpredictable. All comparisons of natural populations to date, that we are aware of, have simply compared populations from constant and variable environments without considering the predictability of those environments (Scheiner, 1993*a*).

The lack of genetic variation for plasticity of r and other life history traits was surprising. Such variation has been found in this species before, including the PA population used here (Lynch *et al*., 1989; Spitze *et al*., 1991; Spitze, 1992). Our husbandry methods may be partially responsible. We used a food level 20% that of Lynch *et al*. (1989); as a result our animals were *c*. 20% smaller at maturity with clutch sizes 60% smaller (compare means for sexuals in Table 4 with table C1 of Spitze, 1992). Also, the mortality suffered during the phenotypic assessment may have led to an underestimate of the genetic variation in the initial populations. However, this mortality had little effect on our estimate of the final population phenotypes and, thus, our conclusions about selection on plasticity are robust.

Another experiment performed with 15 of these clones (Yampolsky & Scheiner, unpublished data) found similar levels of genetic variation for these traits and their plasticities in response to temperature. That experiment did not suffer the mortality problems of this one, and so the estimates of genetic variation are more robust. Thus, again we are confident in our conclusion that the response to selection by plasticity was very weak because of a lack of suitable genetic variation.

One comparable experiment to ours has been performed. Reboud & Bell (1997) examined the

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effects of spatial and temporal variation in light on *Chlamydomonas*, a unicellular green alga. As in our experiment, replicate lines were genetically identical at the beginning of the experiment. The experiment consisted of four treatments – constant light, constant dark, both light and dark (spatial variation), and alternating cycles of light and dark (temporal variation) – with two replicate lines per treatment. Their spatial variation treatment acted like our Unpredictable treatment, while their temporal variation treatment is equivalent to our Predictable treatment. The first three treatments proceeded for 1 yr, or approximately 750 generations in the light treatment and 250 generations in the dark (Bell & Reboud, 1997). Then, the fourth treatment was added and evolution proceeded for an additional 100–200 generations. Selection in the spatially variable environment resulted in the evolution of genetic specialists. Temporal variation, in contrast, selected for phenotypic plasticity. These results are in accord with theoretical predictions, unlike our experiment.

Why the discrepancy? Most likely the difference in outcomes was due to differences in population sizes and the number of generations. In our experiment, the outcome was limited by the initial genetic variation. In contrast, the *Chlamydomonas* experiment had conditions favouring the accumulation of new genetic variation and longer-term evolutionary responses. These differences reinforce our conclusion that the evolution of plasticity will be a long-term response.

(ii) *Plasticity and reproductive mode: goal two*

Clones with different reproductive modes (strictly parthenogenetic *v*. cyclically parthenogenetic) were not differentially adapted to various patterns of environmental variation. The test of this conjecture differs from the one outlined above because, rather than looking to the outcome of a selection experiment, we were assessing previous patterns of adaptation to natural environments. Thus, one potential flaw in our methodology was a failure to sample natural populations in a representative manner. Certainly our requirement that each genotype be electrophoretically unique led to a non-random pattern of sampling. The asexual genotypes were mostly from different ponds as each pond usually held only one or very few clones. On the other hand, the sexual genotypes all came from a single population. Thus, sampling of the latter reproductive type was more geographically limited. A wider sampling of sexual genotypes may have resulted in a different outcome. We did find morphological differences between the reproductive types, but these differences failed to translate into life history differences. These results are somewhat surprising given the typical correlation between adult size and reproductive rate in daphnids (e.g. Yampolsky & Ebert, 1994). A previous comparison of sexual and asexual populations in this region found such a correlation (Lynch *et al*., 1989).

The simplest explanation for our results is that sexual and asexual genotypes are equally able to adapt to the environments of small ponds in northern Illinois and Indiana. This region happens to be a transition between primarily asexual populations to the east and north and primarily sexual populations to the west (Innes *et al*., 1986; Hebert *et al*., 1988). Hebert *et al*. (1993), in documenting this pattern, were unable to find any environmental change that accounted for this transition. They found the opposite geographic pattern with the closely related species *D. pulicaria* (Cerný & Hebert, 1993). Our results suggest that the location of the transition may be due strictly to historical factors, a conclusion echoing that of Hebert *et al*. (1993).

Perhaps our result came from sampling all genotypes from similar types of small ponds. However, *D. pulicaria* did not differ in reproductive type among populations from habitats differing in environmental stability, lakes *v*. ponds (Cerný & Hebert, 1993). On the other hand, *D. pulex* exhibited a consistent south to north shift from sexual populations in the United Kingdom to asexual populations in northern Sweden (Ward *et al*., 1994). This shift, however, was accompanied by a change in ploidy: diploid in the south and polyploid in the north. Polyploidy is usually associated with a switch to strictly asexual reproduction in *Daphnia* (Weider *et al*., 1987; Dufresne & Hebert, 1994). Thus, correlates of reproductive type and various environmental gradients may be due to selection on ploidy level. A more complete test of Scheiner's (1993*a*) conjecture on differences in plasticity between sexual and asexual lineages will require a more widespread comparison between populations of different reproductive types from a variety of habitats.

(iii) *Genetic variation and environmental variation: goal three*

We found inconsistent differences in the amount of genetic variation maintained in constant, predictable, and unpredictable environments (Fig. 2). Previous experiments found that temporal or spatial environmental variation tended to retard the loss of genetic variation compared with constant environments (Powell, 1971; McDonald & Ayala, 1974; Minawa & Birley, 1978; Powell & Wistrand, 1978; Oakeshott, 1979; Mackay, 1980; Haley & Birley, 1983; Bell, 1997; Bell & Reboud, 1997; Reboud & Bell, 1997). In general, spatial variation was more successful than temporal variation in maintaining genetic variation. Thus, our results tend to confirm previous conclusions

that temporal variation acts only weakly to preserve genetic variation (Hedrick, 1986).

(iv) *Conclusion*

Our study failed to find evidence that populations adapt to environmental variation by increasing phenotypic plasticity. These results are consistent with the contention of Via (1993) that selection will mostly act on trait means within environments and that plasticity will evolve strictly as a correlated trait. Recent theoretical work supports this contention by showing that plasticity evolves as a function of its global fitness across environments, a process that is weaker than local fitness responses within environments (Zhivotovsky *et al*., 1996; Scheiner, 1998). However, these results should not be taken to imply that selection can never act directly on plasticity. Rather, we need to understand the balance between local selection on trait means and global selection on trait plasticity. Over evolutionary time even slow processes can be important, as demonstrated by the experiment of Reboud & Bell (1997). Given the likelihood that plasticity will evolve over these longer time periods, perhaps comparative studies need to shift from differences among closely related local populations to those of more distantly related populations or even among different species.

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