

Mutational divergence of life-history traits in an obligate parthenogen

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Three lines of obligately parthenogenetic *Daphnia* were allowed to diverge for a 4-year period (approximately 150 generations) with mutation as the sole source of variability. Life-history traits and morphological characters were then surveyed for between-line differences. Significant divergence was found with respect to both number and size of offspring, with no difference in total offspring biomass. No significant differences were found in any of the other characters. These results confirm the hypothesis that purely asexual lines can accumulate enough polygenic variation via mutation to support potentially adaptive changes on a microevolutionary time scale.

Key words: *Daphnia*, life-history trait, mutational divergence, microevolution.

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Trois lignées de *Daphnia* parthénogène obligé ont eu la possibilité de subir des divergences durant une période de 4 années (environ 150 générations) par mutations comme seule source de variabilité. Les traits vitaux historiques et les caractéristiques morphologiques ont fait l'objet d'examen suivis pour déceler les différences entre les lignées. Des divergences significatives sont survenues quant aux nombres et aux dimensions des descendants, sans toutefois que les biomasses totales des descendants présentent des différences. Aucune différence significative n'a été observée pour l'un ou l'autre des caractères. Ces résultats confirment l'hypothèse que les lignées vraiment asexuées peuvent accumuler assez de variations polygéniques par mutations pour supporter des changements potentiels d'adaptation sur une échelle microévolutive.

Mots clés : *Daphnia*, asexualité, traits vitaux historiques, divergences mutationnelles, microévolution.

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Introduction

The argument has been made frequently that in the absence of recombination as a source of new genetic variation and as a mechanism for the elimination of deleterious mutations, asexual lineages will inevitably end in extinction (Muller 1964; Mayr 1970; White 1973; Maynard Smith 1978). An assumption of the evolutionary dead-end hypothesis is that no selectively advantageous mutations arise within asexual lineages. Lynch and Gabriel (1983) argued that polygenic mutation is an important source of useful variation in asexual organisms and showed that the rate of phenotypic evolution in a large asexual population could match that of an otherwise comparable sexual population if the mutation rate of the former were inflated by twice the effective number of loci for the character. This critical factor refers to characters with an additive genetic basis. It is expected to be lower with nonadditive gene action, since asexual clones can permanently transmit dominance and epistatic effects to their offspring, while sexual parents cannot.

A short-term divergence experiment was performed by Lynch (1985) to determine the rate of polygenic mutation in an obligately parthenogenetic clone of *Daphnia pulex*. Mutations were allowed to accumulate for 2 years (approximately 75 generations) in lines from a single stem mother. Compelling evidence for the appearance of new genetic variation for life-history characters was found. The results reported here supplement the earlier study of Lynch (1985), providing further evidence that obligate parthenogens can

mutationally generate significant amounts of genetic variance for fitness characters in relatively short periods of time.

Materials and methods

Three isolated lines (*X*, *P*, and *S*) of the same obligately parthenogenetic clone of *Daphnia pulex* used by Lynch (1985) were maintained in isolation for 4 years (approximately 150 generations) prior to this experiment. Only one of these lines (line *X*) was represented in the earlier polygenic mutation rate experiment. The other two lines were maintained as isolated stock populations in separate laboratories (line *P* in Plön, Germany, and *S* in Champaign, Illinois). During the 4-year separation, the two stock lines (*P* and *S*) were sustained in jars on artificial pond medium and fed at irregular intervals. Line *X* was maintained in small beakers for the first 2 years as described by Lynch (1985), but for the following 2 years was maintained in jars like the stock population. Because dozens of individuals were simultaneously present in the stock cultures there was plenty of opportunity for selection on newly arisen mutations. Therefore, the following analysis is simply a test of the evolutionary divergence of lines and cannot provide unbiased estimates of the rate of polygenic mutation.

The randomized experimental design used here was quite similar to that of Lynch (1985) but included a few changes in protocol. Twelve sublines for each of the three lines were produced by isolating offspring of a single female from each line. These 12 sublines were maintained for three generations by transferring first-clutch progeny only. Such treatment ensures that maternal, grandmaternal, and great-grandmaternal environmental effects do not contribute to estimates of the between-line variance (Lynch

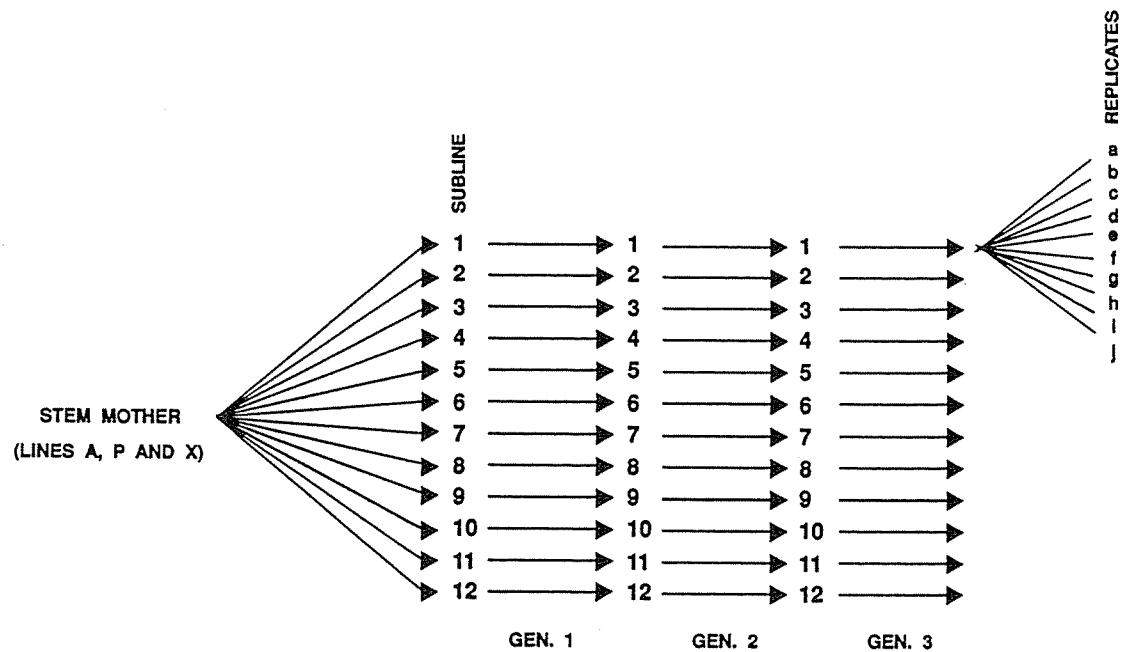


FIG. 1. Schematic diagram of experimental design. One of three stem lines, the 12 sublimes produced from a stem mother and the 10 replicates from each of the 12 sublimes are indicated. Arrows indicate release of progeny from the previous generation.

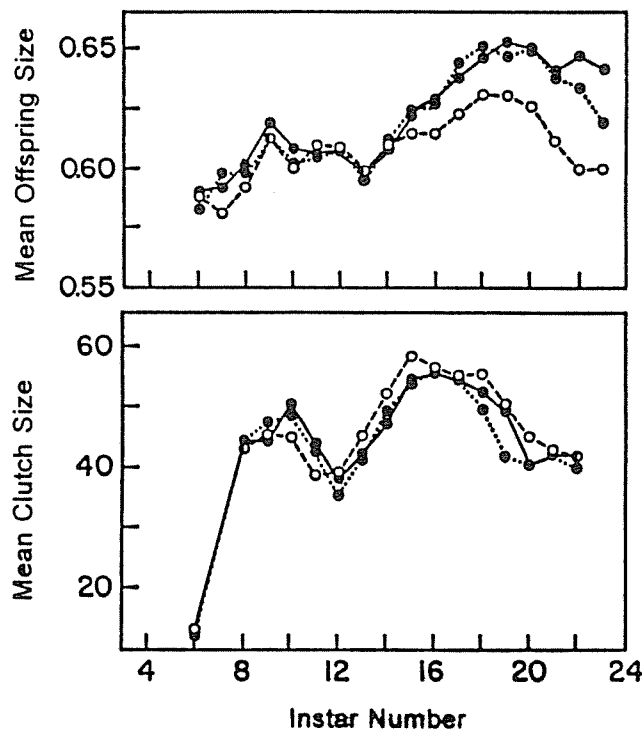


FIG. 2. Mean age-specific offspring sizes and clutch sizes for lines X (—), P (---), and S (···).

1985). Second-clutch progeny were used to produce 10 replicates from each of the 12 sublimes (Fig. 1). Second-clutch progeny are greater in number than first-clutch progeny and were used to ensure an adequate number of replicates. Fortunately, all of the replicates were started on the same day, thus eliminating the need to factor out date effects in the final ANOVA. The food included only *Scenedesmus* as opposed to the *Scenedesmus* and *Chlamydomonas* mixture used by Lynch (1985). All food was recycled daily by adding fresh *Scenedesmus* to restore the concentration to 4.2×10^5 cells/mL. Each day, 4 L of fresh

medium were added to the pooled medium to keep it fresh without causing the animals any unnecessary chemical shock. Two hundred millilitres of food per individual was used as opposed to the 40 mL previously used by Lynch (1985). An excess of food was expected to reduce the magnitude of maternal effects (Lynch and Ennis 1983).

Growth and reproductive data (offspring size and number) were gathered daily for the entire lifespan of each animal to detect a possible increased accumulation of mutational changes for characters expressed at later ages. In theory, selection should be less effective in altering the frequency of mutations that are only expressed late in life and have little influence on fitness (Hamilton 1966).

The individual growth trajectories were summarized by two parameters, a and L_{\max} , obtained by nonlinear least-squares analysis of the equation $L(x) = L_{\max} - (L_{\max} - L_0)^{ax}$, where $L(x)$ is the length at age x and L_0 is the length at birth. L_{\max} represents the plateau of the curve and a defines the "steepness" of the approach to the asymptote. As well, total clutch biomass was quantified by extrapolating weight from length measures using the relation $B = a_1 L^{a_2}$ from Lynch (1986) where estimates of a_1 and a_2 are $\ln(a_1) = 1.874 \pm 0.005$; $a_2 = 2.632 \pm 0.032$ (mean \pm SE) (Lynch 1989).

In addition to the life-history characters, several morphological traits were measured. The offspring from the sixth clutch of the experimental individuals were saved and maintained in jars containing the same type of food used for the experiments. Once these offspring reached maturity, they were preserved in a formalin-sucrose solution. Measurements were taken on the distal and proximal portions of the abdominal setae, and the teeth of the proximal and mid-pectens of the post-abdominal claw were counted as were the post-abdominal spines.

Results and discussion

Sufficient data for analysis of size and reproduction were available for the first 22 instars. None of the age-specific lengths, nor the growth parameters a and L_{\max} , exhibited significant between-line differences. There were no significant between-line differences in any morphological characters.

TABLE 1. Broad-sense heritabilities (between-line variance/total variance) for age-specific offspring sizes and numbers

Instar	Length	Number	Total offspring biomass
6	-0.02	-0.01	-0.00
7	0.09**	-0.01	-0.01
8	0.03*	-0.01	0.00
9	0.01	0.03*	0.00
10	0.03*	0.02	0.05**
11	-0.01	0.02*	0.00
12	-0.02	0.01	0.01
13	0.00	0.02*	0.03*
14	-0.01	0.02	0.00
15	0.01	0.01	-0.01
16	0.06**	-0.01	-0.00
17	0.13**	-0.01	0.01
18	0.13**	0.07**	0.01
19	0.14**	0.08**	0.02
20	0.10**	0.01	-0.01
21	0.06	-0.02	-0.01
22	0.23	-0.01	0.03

NOTE: ** and * denote significance at the 0.01 and 0.05 levels as indicated by *F* tests.

There were, however, significant differences in offspring size and number. These differences were caused primarily by mutation(s) in line *P* that resulted in a dramatic reduction in offspring size in instars 15 and beyond (Fig. 2). Accompanying the reduction in offspring sizes produced at late ages were increases in the clutch sizes of line *P*. Consequently, when offspring lengths were converted to weights by the regression formula for this clone discussed earlier, and the total clutch biomass computed as the product of offspring number and weight/offspring, the differences between lines largely disappeared. Thus, the mutation(s) that arose in line *P* appear to have negative pleiotropic effects on offspring size and number, consistent with the tradeoff often assumed in life-history theory.

The dramatic increase in broad-sense heritability (H^2 , where $H^2 = V^G/V^T$; V^G is genetic variance and V^T is total phenotypic variance) for offspring size with maternal age is qualitatively consistent with Hamilton's (1966) hypothesis that selection should be less effective in altering the frequencies of mutations expressed dominantly at late ages (Table 1). Too much should not be made of this pattern, however, unless offspring size is a more important determinant of fitness than is clutch size, since the latter character exhibits the pattern only weakly. As well, one early instar, seven, did in fact produce a large heritability estimate for offspring size, weakening the general trend.

Evidence for the selective elimination of mutations from the lines during their mass culture is obtained by compari-

son with the earlier results of Lynch (1985). In that study, a large number of lines were maintained as individuals so there was no opportunity for fecundity selection. The mean value of the rate of increase of the between-line variance scaled by the within-line variance was 0.0017 ± 0.0003 per generation for the same life-history characters examined in this study but expressed only up to the third adult instar. For the current study, the standardized divergence rate averaged over all characters is 0.00010 ± 0.00004 , or 3% of the previous value. Even for age-specific offspring sizes, which exhibit the greatest genetic variance of any of the characters, the mean rate is only 0.00045 ± 0.00014 . The smaller divergence rate observed in this study may be due to the reduced opportunity for drift within lines. Since, unlike the protocol in Lynch (1985), these lines were maintained in mass culture rather than as isolated individuals, newly arisen mutations may have been eliminated by drift instead of conserved in separate lineages. As well, stabilizing selection within cultures could have led further to a reduction in between-line variance.

In summary, over a period of only 4 years, isolates of an obligately asexual clone of *Daphnia* have accumulated significant genetic variation for life-history characters. Since small or large offspring sizes may be favoured by natural selection depending on the ecological setting (Lynch 1980), it follows that polygenic mutation is an important source of adaptively significant variance on the microevolutionary time scale.

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