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THE DISTRIBUTION OF LIFE-HISTORY VARIATION IN THE *DAPHNIA PULEX* COMPLEX

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Abstract.—In the midwestern United States the *Daphnia pulex* complex consists of a mosaic of sexual and asexual populations, providing a useful model system for studying the evolutionary forces underlying the maintenance of sex. One asexual and two sexual populations were surveyed for genetic variation for isozymes, mitochondrial DNA, and life-history characters. While the sexual populations exhibited substantial levels of genetic variance for fitness characters, no variation was detected in the asexual population at any level. However, a parallel survey among asexual clones derived from other ponds revealed large amounts of quantitative variation among clones, even among those with the same molecular profile. As a group, the asexuals are more variable for life histories than are the sexual populations. The molecular data indicate a relatively recent origin for the extant asexual *D. pulex*. The polyphyletic origin of these clones, combined with their microevolutionary potential, provides an explanation for their broad geographic distribution. The distribution of sex in the complex cannot be explained with the standard models that assume an invariant asexual population in reproductive isolation from the parental species. Although the frequency of asexuality may be driven by the spread of a sex-limited meiosis suppressor through sexual populations, the complete displacement of sexuality may be prevented by ecological distinctions between the two classes of individuals. On average, the asexuals are larger but produce smaller clutches than the sexuals.

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Many species complexes are now known in which obligately parthenogenetic clones coexist on a regional basis with their sexual ancestors (Bell, 1982; Lynch, 1984a; Suomalainen et al., 1987). Such assemblages provide a useful resource for evaluating the numerous hypotheses regarding the mechanisms responsible for the maintenance of sexuality (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Michod and Levin, 1988). Most of the existing studies on sexual-asexual complexes have been concerned primarily with documenting the number of clones and their geographic range relative to the sexual species. The results of such surveys suggest several explanatory hypotheses for the phylogenetic and geographic distribution of parthenogenesis (Lynch, 1984a).

Our work focuses upon the North American complex of *Daphnia pulex*. While it was long believed that all *Daphnia* were cyclical parthenogens, it is now known that

many clones of *D. pulex* have no sexual phase in their life cycle. In Canada, almost all populations consist of a few (usually less than five) obligately parthenogenetic genotypes (Hebert and Crease, 1980, 1983; Weider and Hebert, 1987; Weider et al., 1987). Just south of the Great Lakes, cyclically parthenogenetic populations become more common, although obligate parthenogenesis is still the dominant reproductive mode (Hebert et al., 1988). Farther south, in central Illinois and Iowa, there is a mosaic of population types ranging from single obligate parthenogens to pure cyclical parthenogens and including various mixtures of both types; cyclical parthenogenesis is common in this region (Lynch, 1983, 1984b; Innes et al., 1986).

At present, we are unable to explain this striking latitudinal gradient in the incidence of sex. One possible explanation is the existence of a dispersal barrier to the northerly expansion of cyclical parthenogens, but this seems questionable, since some pockets of cyclically parthenogenetic populations have been located in Ontario (Hebert et al., 1988). Hebert (1981) has suggested that males produced by some obligate parthenogens may spread obligate asexuality through populations of cyclical parthenogens by transmit-

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ting a dominant gene for sex-limited meiosis suppression. Evidence now exists that this can occur (Innes and Hebert, 1988), although the genetic mechanism is more complicated than was first suggested. It can also be stated confidently that obligately parthenogenetic lineages of *Daphnia pulex* have a polyphyletic origin (Crease et al., 1989), consistent with Hebert's (1981) hypothesis. It is unclear why the conversion to asexuality is nearly complete in the north but not in the south.

The purpose of this study was to evaluate whether there are any fundamental differences in the life-history properties of obligate parthenogens (hereafter termed asexuals) and cyclical parthenogens (hereafter referred to as sexuals) that might explain their differential distribution. Two electrophoretically diverse sexual populations and one asexual population that is fixed with respect to isozyme and mitochondrial-DNA (mtDNA) markers were assayed for levels of quantitative-genetic variation to obtain some insight into their microevolutionary potential. Several additional and unique asexuals were included in the study to see whether any generalizations could be made regarding their life histories. A parallel survey of isozyme and mtDNA variation provides some insight into the phylogenetic affinity of the members of the complex and its relationship to life-history similarities.

MATERIALS AND METHODS

All of the clones in this study were obtained from sites in east-central Illinois and west-central Indiana (Fig. 1). Populations PA, KA, and TS were the sources of animals for the quantitative-genetic experiments. Population PA was obtained from a natural oxbow pond in Fountain Co., IN, near Portland Arch State Nature Preserve. The pond, which dries up each year from late June to late July, has a maximum depth of 1 m. Important predators are the dipteran larvae *Mochlonyx* (March–April) and *Chaoborus* (from mid-April to drying) and *Ambystoma* salamander larvae (from late April to late June). Population KA inhabits a temporary pond in Kickapoo State Park, Vermilion Co., IL. The pond, which is located in an old strip-mine area, was created by human disturbance early in this century. Maximum

depth is 2 m, and complete desiccation occurs annually from August to September. *Chaoborus*, notonectids, and dytiscid larvae are present but not abundant. Population TS was obtained from a permanent pond (maximum depth = 1 m) approximately 500 m from the KA site. *Daphnia* appear to inhabit the pond throughout the year. *Chaoborus* are always fairly abundant. Notonectids and dytiscid larvae are sometimes present in low numbers.

In order to sample maximum levels of genetic variance for quantitative traits, the field collections were made shortly after the spring ephippial hatch (Lynch, 1984b). All clones were acclimated to the experimental conditions for at least one generation prior to life-table analysis. At the outset, two sublines were extracted from each clone, and progeny from the second or third clutch from each subline were used in the experiment. This protocol insures that environmental maternal effects do not contribute to the between-clone component of variance in the final analysis (Lynch, 1984c). All experiments were performed at 20°C, with a 12L:12D photoperiod, at a food density of 3×10^5 *Scenedesmus* cells ($\sim 2.6 \mu\text{g C}$) per milliliter. Each animal was provided with 140 ml of fresh medium every day.

The PA survey involved 79 clones, which were isolated from the pond in mid-April 1986 and assayed in two experiments. In the first experiment, two individuals were measured per subline, whereas the second experiment employed no replication within sublines. In the first experiment, the water supply consisted of initially autoclaved pond water kept in a sterilized plastic garbage pail. This water was partially recycled between food changes, a procedure that eventually led to a dense growth of filamentous fungi in the water supply. In order to avoid this problem in the second experiment, an alternate procedure was followed. Water from a large trough with an active population of *Daphnia* was filtered through two layers of Whatman #1 filter paper as needed.

We analyzed 120 KA clones (collected in the field from early April to early May) and 136 TS clones (collected in early April) together in two blocks in 1987. Half of the clones were utilized in each block, each clone being represented by a single offspring from

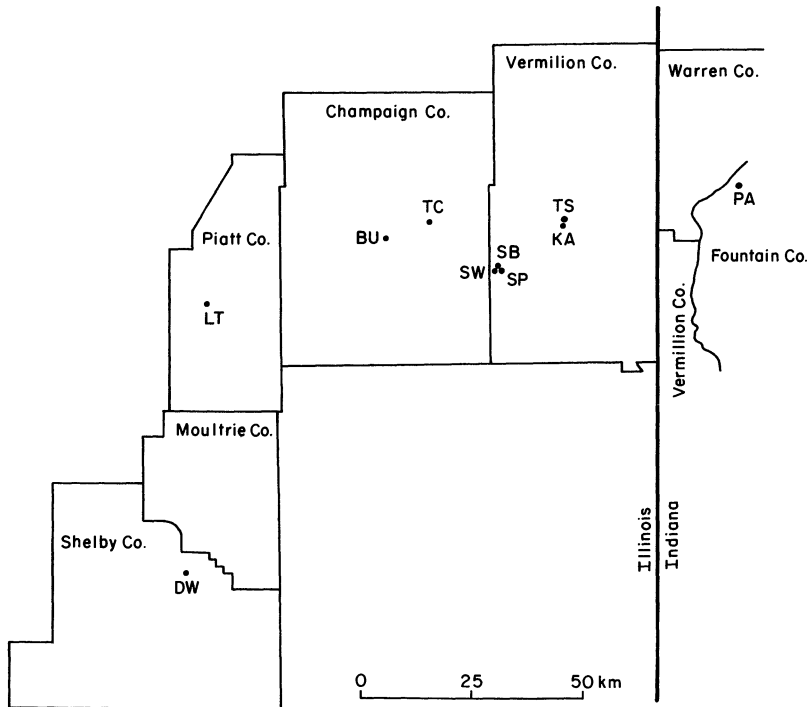


FIG. 1. Map of several counties in Illinois and Indiana, showing field sites (abbreviated as in text). PA, KA, and TS were the three main study populations.

each of two sublimes. All aspects of these experiments were essentially the same as those for the second PA experiment. In the second block of the 1987 experiment, we included 11 obligately parthenogenetic clones obtained from various sites in central Illinois (Fig. 1); each of these clones was represented by single individuals from each of ten sublimes.

During the life-table experiments, all individuals were measured daily from the base of the tail spine to the top of the head. Eggs in the brood chamber were counted, as were the offspring released, and five random individuals from each newly released brood were measured. In order to improve the accuracy of estimates of age at reproduction, 11 egg development stages were distinguished, based on embryonic morphology. The durations of these stages were established by laboratory observations. Thus, rather than recording the 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 from the time at which a released clutch was first

observed. This indirect approach to estimating age at reproduction is possible because *Daphnia* extrude a new clutch into the brood chamber almost immediately upon the release of the previous brood.

The data were analyzed by standard ANOVA procedures. Estimates of variance components were extracted from the observed mean squares, and large-sample variances of these components and of the broad-sense heritabilities were obtained by use of Taylor-expansion formulations (Lynch, 1984c). The first PA experiment was analyzed by nested ANOVA (clones, sublimes within clones, replicates within sublimes), and the environmental variance was estimated as the sum of the variances at the two lower levels. The second PA experiment was analyzed as a simple one-way ANOVA. Since each clone was represented by single individuals from two sublimes, all of the environmental variance (including that caused by maternal effects) appeared in the within-clone component of variance. A similar analysis was performed on the KA and TS data sets, except that both blocks were analyzed simultaneously, so clones

were nested within experiments. The between-experiment component of variance, which was always of minor importance, was not included in the estimate of environmental variance.

In order to avoid an artificial inflation of the environmental-variance estimates (and consequent reduction in the heritabilities), we removed the fraction of this variance that was attributable to measurement error. For body-size measurements, for example, many pairs of measures within individuals were available for each instar. Most instars last for two days, during which time the size of the carapace does not change. The average variance of such paired measures within an instar, divided by the mean number of measures per instar, was used as an estimate of the variance of instar-specific sizes caused by imprecise measurement. Measurement variances for ages at reproduction were obtained by following a cohort of 40 adult individuals for 1.5 days at 0.05-day intervals and comparing the actual release times with those estimated by the egg development-stage protocol. Offspring number was assumed to be measured without error.

On several occasions, the three main study populations were assayed for isozyme variation at seven loci using starch-gel (Lynch, 1983) and cellulose-acetate (Hebert and Payne, 1985) electrophoresis. All of the clones used in the life-table experiments were also assayed for their multilocus genotypes in order to look for linkage disequilibrium between electrophoretic markers and quantitative-trait loci. No evidence for such associations was found.

The mitochondrial genotypes of a sample of isolates from each of the three study populations, as well as of each additional obligately parthenogenetic clone, were determined by Southern blot analysis of restriction-site variability. Twenty-two restriction enzymes with five or six base-pair recognition sequences were used. Technical details of the protocol are given in Crease (1986).

RESULTS

Isozyme Variation.—The isozyme data are consistent with the hypothesis that the

KA and PA populations consist entirely of randomly mating cyclical parthenogens. Genotype frequencies for four polymorphic loci (*Pgm* and *Pep* [1981–1986]; *Fum* and *Mpi* [1986 only]) in the PA population never deviated significantly from Hardy-Weinberg expectations. Samples at the *Pgm*, *Pgi*, and *Got* (all 1984–1987) loci and at the *Fum* locus (1986–1987) in the KA population were also consistent with Hardy-Weinberg expectations (only one significant deviation at the 0.05 level in 28 comparisons).

Some rather striking differences exist between these two populations (Table 1). The KA population is fixed for a single allele at two loci (*Mpi* and *Pep*) and nearly fixed at two additional loci (*Fum* and *Pgm*), all of which exhibit high heterozygosity in Portland Arch Pond. Although the PA population is fixed at two loci (*Pgi* and *Got*) for which the KA population is polymorphic, the net result is that the mean heterozygosity in the latter (0.11 ± 0.04) is approximately one-third that of the former (0.31 ± 0.10). These are biased estimates of total genomic heterozygosity, since we have avoided the use of typically monomorphic loci in our surveys, but they should not be biased with respect to each other.

The TS population presents a rather different picture, since all observed individuals are identical at all assayed protein loci (Table 2). Three of these loci have exhibited fixed heterozygosity over several years, and progeny from laboratory-hatched ephippia show no evidence of recombination. Thus, it is clear that the TS population is reproducing by obligate parthenogenesis, unlike the PA and KA populations, which engage in a bout of sex each year.

Although the TS genotype has not been found in any other local pond, four other distinct groups of obligate parthenogens have been discriminated on the basis of isozymes within our sampling area (Table 2). All of these isozyme genotypes occur in at least two ponds. In all cases, obligate parthenogenesis has been verified by direct observation in the laboratory. All but one of the obligate parthenogens that we have been able to locate in central Illinois are *FS* heterozygotes at the *Ldh* locus. They are therefore "urban" clones, using the notation of Hebert and Crease (1983). The other clone

TABLE 1. Summary statistics for gene frequencies and expected heterozygosities for the six polymorphic enzyme loci observed in the PA and KA populations. *N* is the number of individuals assayed per locus.

Locus	Population	<i>N</i>	Alleles				Heterozygosity
			<i>F</i>	<i>M</i>	<i>S</i>	<i>S</i> ⁻	
<i>Pgm</i>	PA	529	0.491	0.106	0.403	—	0.585
	KA	517	0.075	0.925	0.000	—	0.139
<i>Pgi</i>	PA	529	0.000	1.000	0.000	—	0.000
	KA	555	0.047	0.847	0.106	—	0.269
<i>Got</i>	PA	529	—	1.000	—	0.000	0.000
	KA	562	—	0.955	—	0.045	0.086
<i>Pep</i>	PA	529	—	0.653	0.347	—	0.453
	KA	567	—	1.000	0.000	—	0.000
<i>Fum</i>	PA	98	—	0.464	0.536	—	0.497
	KA	84	—	0.095	0.905	—	0.172
<i>Mpi</i>	PA	97	—	—	0.784	0.216	0.339
	KA	128	—	—	1.000	0.000	0.000

is an *SS* homozygote and is therefore a “forest” clone.

Mitochondrial DNA.—Of the 83 restriction sites revealed in this study, 16 were variable. Nine mtDNA genotypes were distinguished (Fig. 2). Two of these were restricted to the sexual populations, while six were restricted to asexual individuals, and one appeared in both types of individuals. All 21 of the isolates from KA had mtDNA profile 15. Four the PA isolates had mtDNA genotype 7, while 15 had mtDNA genotype 14. These two genotypes differed from each other at five sites and from the KA genotype by seven and six sites, respectively. The mtDNA genotypes of the asexuals are no more distant from each other or from the sexuals than the latter are from each other. This is consistent with results from Ontario clones (Crease et al., 1989) and implies that the asexual lineages are of recent origin.

Whereas the isozyme survey identified only five asexual genotypes, the additional

information for mtDNA indicates that this is an underestimate of the true number. Isolates of the widespread isozyme genotype 4 all have the same mtDNA genotype, but isozyme genotype 2 has two mtDNA variants (Table 2), which differ at two sites, possibly due to mutations subsequent to the origin of an asexual lineage.

Mean Phenotypes.—When KA is compared to the average of the two PA assays, the mean instar-specific sizes for the two sexual populations are found to be very similar (Fig. 3). The asexual individuals from TS, on the other hand, are relatively small; from instar 4 onwards, they are 0.2–0.3 mm smaller, on average, than the sexual individuals. With the exception of DW4,1 (individuals from site DW with isozyme genotype 4 and mtDNA genotype 1) the remaining obligate parthenogens are all larger than the average sexual individual through instar 6, often by as much as 0.1 mm.

TABLE 2. Multilocus genotypes for obligately parthenogenetic *Daphnia pulex*. The first digit for clone number refers to the isozyme genotype, while the second refers to the mtDNA genotype.

Clone designation			Genotypes for protein loci					
Isozyme	mtDNA	Site	<i>Pgm</i>	<i>Pgi</i>	<i>Got</i>	<i>Pep</i>	<i>Fum</i>	<i>Ldh</i>
1	5	TS	<i>MS</i> ⁼	<i>MM</i>	<i>FM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
2	6	BU	<i>MM</i>	<i>MM</i>	<i>FM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
2	4	LT	<i>MM</i>	<i>MM</i>	<i>FM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
3	2	SB, SW, LT	<i>FF</i>	<i>M⁺M</i>	<i>MM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
4	1	SP, LT, DW, TC	<i>MM</i>	<i>MS</i>	<i>MM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
5	3	SP	<i>FM</i>	<i>MM</i>	<i>MM</i>	<i>MM</i>	<i>SS</i>	<i>FS</i>
6	7	TC	<i>FM</i>	<i>MM</i>	<i>MM</i>	<i>MM</i>	<i>SS</i>	<i>SS</i>

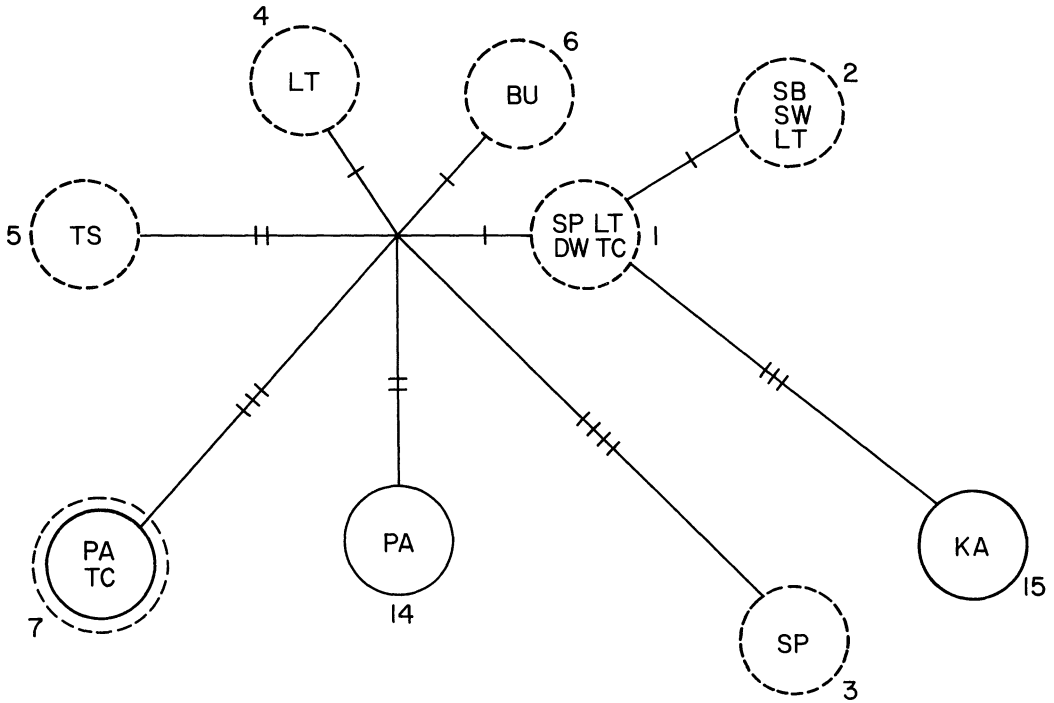


FIG. 2. Unrooted network diagram for the nine mitochondrial genotypes recognized in this study. Each crossbar represents a single restriction-site difference between adjacent genotypes. There are no parallel gains or losses of sites in the network, so the total number of site differences between any pair of genotypes is simply the sum of the differences along the connecting branches. Solid circles represent cyclical parthenogens; dashed circles represent obligate parthenogens. The number outside each circle is the mtDNA genotype number, as used in Crease et al. (1989).

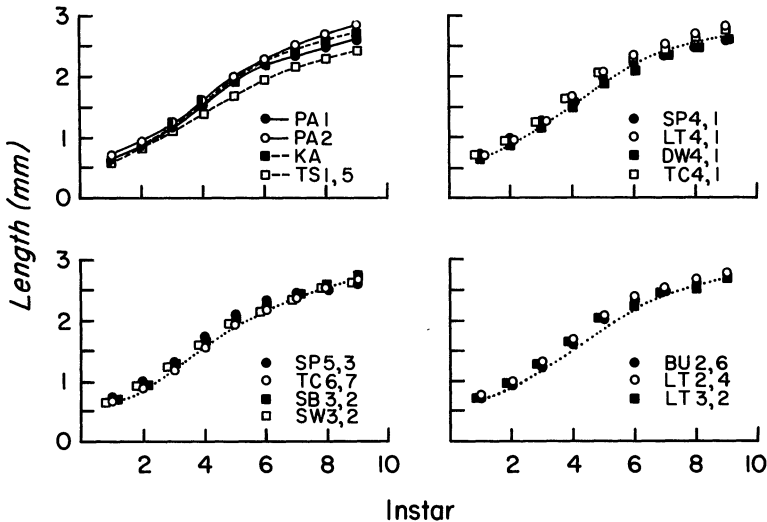


FIG. 3. Mean instar-specific lengths for the two sexual populations (PA and KA) and the asexual clones (abbreviations: TS1,5 = site TS, isozyme genotype 1, mtDNA genotype 5, etc.). PA1 and PA2 refer to the means from the two Portland Arch experiments. For comparative purposes, the KA results are given as dotted lines in the three plots presenting data for the asexual lineages other than TS1,5. Standard errors for the PA, KA, and TS results are approximately 0.005, much less than the diameter of the points. For the remaining asexuals, the standard errors are approximately 0.02, so the 95% confidence limits are approximately half the diameter of the points.

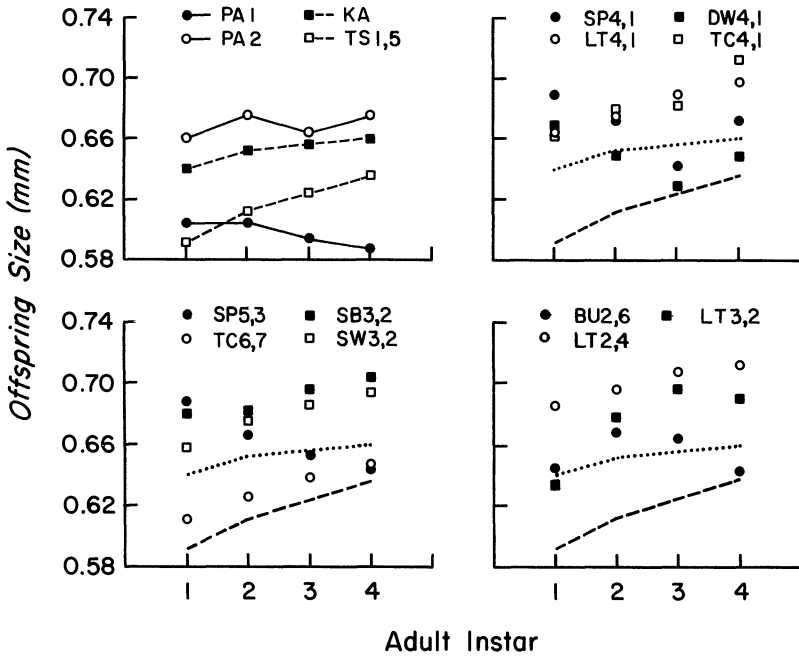


FIG. 4. Mean offspring sizes produced in the first four adult instars for the two sexual populations (PA and KA) and the asexual clones (abbreviations: TS1,5 = site TS, isozyme genotype 1, mtDNA genotype 5, etc.). PA1 and PA2 refer to the means for the two Portland Arch experiments. For comparative purposes, the KA and TS results are given as dotted and dashed lines in the three panels presenting data for the asexual lineages other than TS1,5. Standard errors for the PA, KA, and TS results are approximately 0.002, so the 95% confidence limits are approximately the diameter of a point. For the remaining asexuals, the standard errors are approximately 0.01.

Consistent with the preceding results, the TS asexuals produced much smaller progeny (0.02–0.05 mm smaller) than did the sexuals from KA (Fig. 4). The two PA experiments yielded dramatically different results for offspring size, suggesting that the expression of this character is very sensitive to environmental modification. The mean offspring sizes from all of the remaining asexual lineages are greater than those from TS, and most exceed the sizes for KA, some significantly so.

While age-specific progeny production was similar in both sexual populations, that for the TS asexual population was approximately 60% of that for the KA animals (Fig. 5). All other asexual lineages produced offspring in quantities intermediate to the levels for TS and KA. These differences in progeny production reflect variation in egg production, since egg survivorship did not differ greatly among populations (Table 3). The ages at reproduction are quite similar for both asexual and sexual individuals (Fig. 6).

A few of the asexual clones reproduce at slightly, but significantly, later ages than the average KA individual, but none reproduces significantly earlier.

To test whether the asexual clones clustered with respect to life-history characters in accordance with their molecular affinities, we computed the matrix of pairwise Mahalanobis distances. The results are summarized in Figure 7 in the form of a tree obtained by use of J. Felsenstein's PHYLIP program FITCH. As expected on the basis of the previous results, TS1,5 is the outlying clone, but there is no obvious pattern to the clusters of the remaining clones. Analysis of variance revealed highly significant differences in body sizes and clutch sizes among the four 4,1 isolates obtained from different sites and in ages at reproduction and clutch sizes among the three 3,2 clones. Consequently, there is no correspondence between molecular affinity and life-history similarity.

Developmental Stability.—It has been

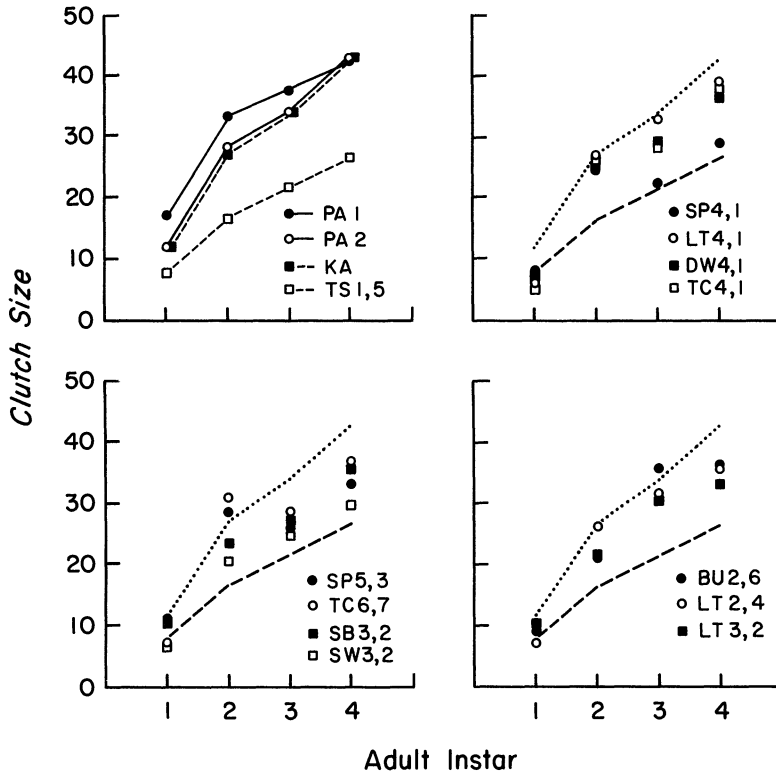


FIG. 5. Mean instar-specific clutch sizes for the two sexual populations (PA and KA) and the asexual clones (abbreviations: TS1,5 = site TS, isozyme genotype 1, mtDNA genotype 5, etc.). PA1 and PA2 refer to the means for the two Portland Arch experiments. For comparative purposes, the KA and TS results are given as dotted and dashed lines, respectively, in the three panels presenting data for the asexual lineages other than TS1,5. Standard errors for the PA, KA, and TS results are approximately 0.5, so the 95% confidence limits are approximately the diameter of a point. For the remaining asexual lineages, the standard errors are approximately 1.5.

suggested that an inevitable consequence of selection on a population of obligate parthenogens in a variable environment is the evolution of generalized genotypes whose fitness is relatively insensitive to environmental modification (Lynch, 1984a). A prediction of this general-purpose-genotype hypothesis is that the environmental component of variance for fitness-related characters will be lower in obligate parthenogens than in cyclical parthenogens. Since macro-environmental sources of variance were excluded by our experimental design, it is not clear that our results are of relevance to the general-purpose-genotype hypothesis. Our estimates of environmental variance (within-clone variance, V_E) are best interpreted as measures of developmental instability in the face of intangible sources of micro-environmental variation.

The pooled estimates of V_E for the PA, KA, and TS populations and for the remaining asexuals yield rather inconsistent results (Table 4). From the standpoint of growth rates and clutch sizes, the clones from sexual populations are equally or more variable than the obligate parthenogens. However, this pattern is less pronounced with offspring size, and there is no clear pattern with age at first reproduction and instar duration.

Heritabilities.—Several generalizations can be made with respect to the broad-sense heritabilities (between-clone variance/total phenotypic variance) for the three populations (Table 5). First, there is no discernible genetic variation for any life-history character in the asexual TS population. Second, there is significant genetic variance for all life-history traits in the sexual populations,

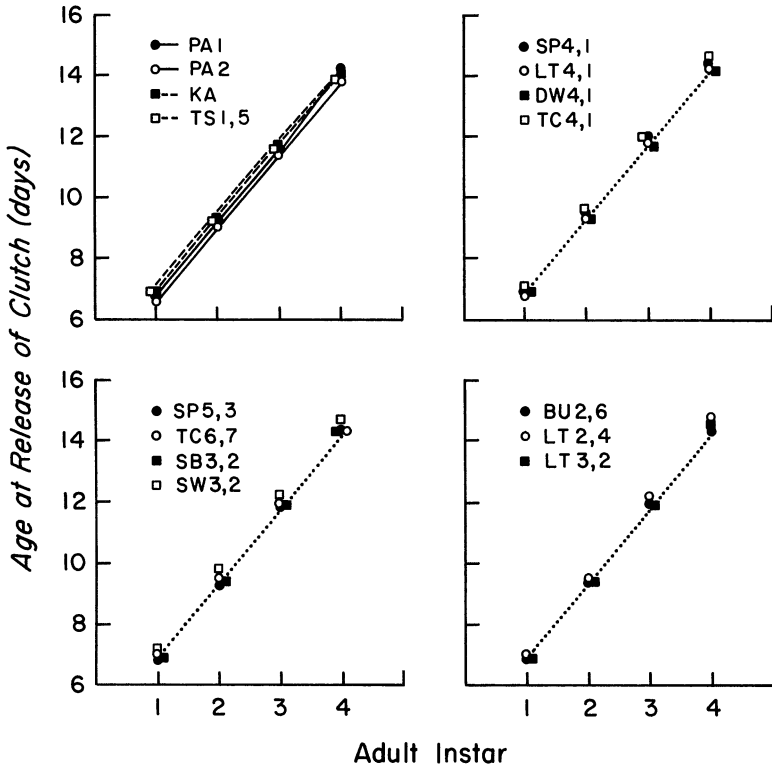


FIG. 6. Mean ages at release of the first four clutches for the two sexual populations (PA and KA) and the asexual clones (abbreviations: TS1,5 = site TS, isozyme genotype 1, mtDNA genotype 5, etc.). PA1 and PA2 refer to the means for the two Portland Arch experiments. For comparative purposes, the KA results are given as dotted lines in the three panels presenting data for the asexual lineages other than TS1,5. Standard errors for the PA, KA, and TS results are approximately 0.04, so the 95% confidence limits are approximately half the diameter of a point. For the remaining asexuals, the standard errors are approximately 0.1.

except for age at first reproduction in PA and instar durations. Third, heritabilities in the KA population tend to be greater than those in the PA population. The former population tends to have higher levels of genetic variance as well as lower levels of environmental variance (Table 4).

Thus, the rankings of the two sexual pop-

ulations on the basis of quantitative-genetic variance are contrary to the rankings based on isozymes and mitochondrial DNA. We have no reason to suspect that stabilizing selection operated more intensely on our laboratory isolates from PA than on those from KA. It is, of course, possible that an expansion of the electrophoretic survey to additional loci would bring the two types of results more in line, but the likelihood of this is low, since the differences at the molecular level are highly significant.

DISCUSSION

The results of this study are consistent with previous work (Lynch, 1984b) in demonstrating that cyclically parthenogenetic populations of *Daphnia* harbor substantial amounts of genetic variation for growth and reproductive rates. The heritabilities for clutch sizes were highly significant and, with

TABLE 3. Mean proportion of eggs that gave rise to live offspring in the first four clutches; values in parentheses are standard errors. The 11 obligately parthenogenetic lineages (other than TS) were pooled to obtain the data shown in the last column.

Clutch	Egg survival		
	KA	TS	Asexuals
1	0.84 (0.02)	0.87 (0.01)	0.79 (0.03)
2	0.93 (0.02)	0.91 (0.01)	0.89 (0.02)
3	0.98 (0.02)	0.92 (0.01)	0.91 (0.03)
4	0.98 (0.02)	0.93 (0.01)	0.95 (0.02)

one exception, in the range of 0.30–0.55. Such levels of heritability are exceptionally high for invertebrates, which have a median heritability of approximately 0.25 for life-history characters and have heritabilities exceeding 0.4 for this class of traits in less than 20% of observed cases (Mousseau and Roff, 1987).

In contrast with the sexual populations, the asexual TS population exhibited no significant genetic variance for any quantitative character. A direct survey of its nuclear and mitochondrial genes did not reveal any variation either. This apparent absence of genetic variation is in contrast to the situation in another population of asexuals (all electrophoretically identical) which yielded estimates of heritability comparable to those of a coexisting sexual population (Lynch, 1984*b*). The results of the present study are much more reliable than the analysis in Lynch (1984*b*), which relied on rather small sample sizes. However, it is conceivable that substantial differences in levels of genetic variation exist among asexual populations of *Daphnia*. All such populations are expected to contain some genetic variance as a simple consequence of polygenic mutation (Lynch and Gabriel, 1983), although the time required for the variation to build up to detectable levels from an initial colonization event can be substantial. With a rate of input of new genetic variance of $V_M/V_E = 0.002$ per generation, a representative value for the characters in this study (Lynch, 1984*c*), it would take in excess of 100 generations (the exact time depending upon the intensity of selection) for the heritability to reach the minimum level of detectability ($H^2 = 0.2$) for our experiments. Since it is unlikely that the *Daphnia* in the TS pond pass through more than 20 generations per year, the initial TS1,5 colonist would have to have arrived at least five years prior to our study for us to have any chance of detecting significant genetic variation. Our records on the pond only extend back four years, but throughout this period TS1,5 has been well-established.

Our survey of obligate parthenogens derived from different ponds provides additional evidence for the ability of asexual lineages to accumulate mutationally derived variation for life-history characters. Small

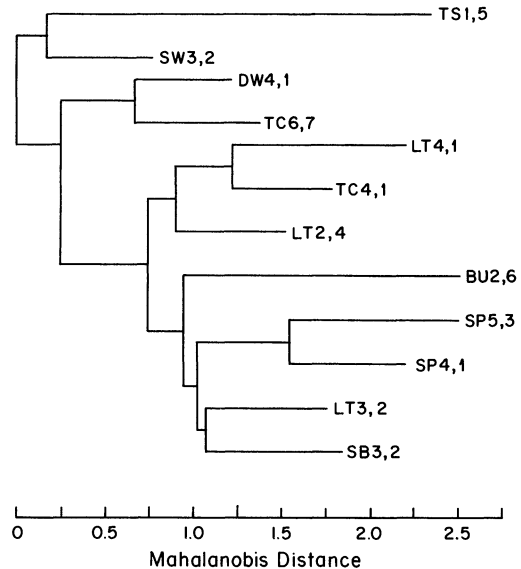


FIG. 7. Mahalanobis distances, based on clonal life histories, fitted by a Fitch-Margoliash procedure. The average percentage standard deviation between observed and fitted distances is 9.3%. Abbreviations: TS1,5 = site TS, isozyme genotype 1, mtDNA genotype 5, etc.

but significant differences appeared between clones SB3,2 and SW3,2 taken from ponds only 800 meters apart. Genotype 4,1 was found in four ponds spread throughout our study area, and the isolates were significantly different in several respects. This particular genotype will be interesting to pursue in future studies, since it appears to extend into Michigan (Hebert et al., 1988) and Ontario (Hebert and Crease, 1983; Crease et al., 1989). At least two other obligate parthenogens in this study may be representatives of widely dispersed lineages. Genotypes identical to 2,4 and 5,3 have also been found in Ontario (Hebert and Crease, 1983; Crease et al., 1989).

Estimates of the rate of mtDNA evolution in various organisms range from ~0.5% to 2.0% nucleotide substitutions per million years (Brown, 1983; Wilson et al., 1985). Thus, since the maximum sequence divergence in this study (1.3%) involves the mitochondria carried by two cyclical parthenogens, it is extremely unlikely that the extant lineages of obligate parthenogens are older than two million years. Indeed, on the basis of the existing data, we cannot rule out the hypothesis that they are substantially young-

TABLE 4. Pooled estimates of the environmental components of phenotypic variance (V_E) and their standard errors (SE) for life-history characters in the two sexual populations (PA and KA), the asexual population (TS), and the other obligately parthenogenetic clones (pooled to obtain the data in the last two columns). ΔB_i = the change in length between instars i and $i + 1$; B_{O_i} = the size of offspring in the i th clutch; C_i = the number of live offspring produced in the i th clutch; k_1 = the age at first reproduction; and D_i = the duration of the i th adult instar.

Trait	PA		KA		TS		Asexuals	
	V_E	SE	V_E	SE	V_E	SE	V_E	SE
ΔB_1	0.00056	0.00012	0.00085	0.00036	0.00012	0.00028	-0.00007	0.00027
ΔB_2	0.00141	0.00031	0.00029	0.00034	0.00001	0.00030	0.00076	0.00038
ΔB_3	0.00224	0.00045	0.00069	0.00022	0.00096	0.00023	0.00048	0.00019
ΔB_4	0.00213	0.00045	0.00074	0.00021	0.00150	0.00028	0.00110	0.00016
ΔB_5	0.00270	0.00038	0.00121	0.00026	0.00187	0.00031	0.00151	0.00022
ΔB_6	0.00230	0.00044	0.00200	0.00039	0.00128	0.00024	0.00078	0.00011
ΔB_7	0.00109	0.00027	0.00122	0.00027	0.00089	0.00018	0.00072	0.00011
ΔB_8	0.00138	0.00059	0.00092	0.00028	0.00052	0.00018	0.00119	0.00020
B_{O1}	0.00054	0.00014	0.00026	0.00005	0.00075	0.00012	0.00103	0.00017
B_{O2}	0.00026	0.00005	0.00034	0.00007	0.00033	0.00005	0.00072	0.00012
B_{O3}	0.00038	0.00007	0.00021	0.00004	0.00043	0.00007	0.00048	0.00007
B_{O4}	0.00054	0.00014	0.00044	0.00008	0.00042	0.00007	0.00071	0.00011
C_1	16.19	2.41	10.26	1.50	4.53	0.57	7.31	1.04
C_2	44.76	7.47	42.87	6.36	16.12	2.08	35.30	5.02
C_3	66.59	13.32	48.07	7.46	21.23	2.74	59.80	8.54
C_4	93.76	24.32	81.10	13.33	24.98	3.25	52.08	7.44
k_1	0.1548	0.0253	0.0656	0.0113	0.2352	0.0312	0.1046	0.0149
D_2	0.0090	0.0051	0.0058	0.0044	0.0068	0.0039	0.0301	0.0044
D_3	0.0054	0.0069	0.0158	0.0061	0.0077	0.0040	0.0123	0.0020
D_4	0.0181	0.0084	-0.0005	0.0038	0.0112	0.0045	0.0042	0.0010

TABLE 5. Broad-sense heritabilities (H^2) for life-history characters in the two sexual populations (PA and KA) and the asexual population (TS). Inequality signs refer to differences in levels of genetic variance that exceed two standard errors. ΔB_i = the change in length between instars i and $i + 1$; B_{O_i} = the size of offspring in the i th clutch; C_i = the number of live offspring produced in the i th clutch; k_1 = the age at first reproduction; and D_i = the duration of the i th adult instar.

Trait	PA		PA-KA comparison	KA		KA-TS comparison	TS	
	H^2	SE		H^2	SE		H^2	SE
ΔB_1	0.26	0.09	>	-0.17	0.31		-0.12	1.02
ΔB_2	0.22	0.09		0.46	0.35		0.78	3.82
ΔB_3	0.22	0.08		0.40	0.14	>	0.02	0.17
ΔB_4	0.27	0.07		0.57	0.10	>	0.07	0.12
ΔB_5	0.30	0.07		0.44	0.09	>	-0.01	0.12
ΔB_6	0.11	0.10		0.32	0.10	>	0.01	0.13
ΔB_7	0.20	0.12		0.29	0.11	>	0.03	0.14
ΔB_8	0.28	0.13		0.40	0.13	>	-0.03	0.21
B_{O1}	0.33	0.14		0.60	0.07	>	0.09	0.12
B_{O2}	0.55	0.06		0.49	0.08	>	0.16	0.10
B_{O3}	0.48	0.07	<	0.74	0.05	>	-0.05	0.13
B_{O4}	0.36	0.11		0.35	0.10	>	-0.04	0.13
C_1	0.17	0.08	<	0.41	0.07	>	0.02	0.09
C_2	0.30	0.07		0.39	0.08	>	0.01	0.09
C_3	0.40	0.08		0.55	0.07	>	0.11	0.08
C_4	0.40	0.08		0.45	0.08	>	0.07	0.09
k_1	0.14	0.09		0.28	0.09		-0.14	0.12
D_2	0.23	0.20		0.30	0.34		0.02	0.39
D_3	0.30	0.29		0.13	0.24		0.33	0.22
D_4	-0.51	1.11		1.09	0.73		-0.22	0.42

ger. This does not eliminate the possibility that obligate parthenogenesis is an ancient reproductive option in *Daphnia*. It only indicates that asexual lineages are relatively short-lived.

Since there are vast numbers of obligate parthenogens in the northern United States and Canada that we have not examined for life-history variation (Hebert et al., 1989), it now seems clear that, as a group, asexual *D. pulex* harbor at least as much genetic variation for quantitative traits as do the sexuals. In central Illinois, the asexuals span the entire range of variation of the sexual members of the complex. This large amount of variation is presumably due to a currently high rate of origin of obligate parthenogenesis relative to the rate of extinction. Hebert et al. (1988) note that the gene-frequency distributions within the asexual and sexual components of the complex are very similar, suggesting that the obligate parthenogens are an approximately random sample of sexual genotypes. Since sexual populations are diverse with respect to quantitative-genetic variation, it then comes as no surprise that equally substantial variation exists among obligate parthenogens of independent origins.

This large amount of variation among asexual lineages and the potential for microevolutionary change within lineages helps to explain the broad geographic distribution of the asexual complex. For most environments, there may exist an asexual genotype that is at least as successful as any sexual genotype. The local dominance of any single obligate parthenogen may be transient owing to fluctuating selection. However, provided that dispersal is not a major barrier to the distribution of asexuals, the regional pool may always harbor suitable replacement variants. Such a scenario is rather different from Bell's (1982) "tangled-bank" model, which states that sexual lineages dominate spatially complex environments by producing genetically diverse progeny capable of occupying most available niches. The tangled-bank model does not apply to complexes in which asexual-sexual hybridizations result in the frequent production of new clones (Bell, 1988).

It is still unclear why cyclical parthenogenesis in *D. pulex* declines to nearly un-

detectable levels at higher latitudes. The results of this study suggest a reason why genes with action similar to Hebert's (1981) dominant sex-limited meiosis suppressor are unlikely to be capable of completely eradicating cyclical parthenogenesis. Obligate and cyclical parthenogens are not ecologically equivalent. In the midwestern United States, the obligate parthenogens are larger on average and therefore more vulnerable to vertebrate predators. They also produce smaller clutches. If, for these or other reasons, the relative fitness of the asexuals tends to be lower than that of the sexuals, an equilibrium must arise eventually from a balance between the production of new asexuals by hybridization with sexuals and the loss of asexuals by natural selection.

There is an additional problem in interpreting the latitudinal gradient in sexuality in *D. pulex* as a simple consequence of the spread of a meiosis suppressor. Superimposed on the sexuality gradient is a gradient within the asexual component of the complex. Hebert and Crease (1983) recognized that asexual *D. pulex* can be separated into two groups on the basis of *Ldh* genotypes: "urban" (*Ldh^{FS}*) and "forest" (*Ldh^{SS}*) clones. Sexual *D. pulex* are always *Ldh^{SS}*. The *Ldh^F* allele in the urban clones is thought to originate from interspecific hybridizations of *D. pulex* with *D. pulicaria*. The distributions of sexual *D. pulex* and the urban clones are similar. They are found in the midwestern United States up into the Great Lakes region but decline to undetectable levels farther north. In contrast, the forest clones are dominant in Canada, from the Arctic southwards into the Great Lakes region. Some forest clones have been found just south of the Great Lakes, but to date only one has been found in the midwest. Thus, the question arises as to whether the latitudinal gradient in the *D. pulex* complex, which superficially appears to be an antagonistic interaction between sexual and asexual reproductive modes, is more fundamentally related to an interaction between a forest-clone complex and a cyclic parthenogen-urban-clone complex.

In closing, we wish to emphasize that the polyphyletic origin and microevolutionary potential of asexual lineages, as well as their incomplete reproductive isolation from their

sexual ancestors, are by no means unique to *Daphnia*. They are properties of the majority of parthenogenetic complexes of animals (Lynch, 1984a). Yet nearly all existing models on the adaptive significance of sex fail to recognize these realities (see Michod and Levin [1988] for the latest review). Since the ecological and evolutionary implications of these complexities are by no means trivial, there is reason to question the general relevance of much of the current theory on the phylogenetic and geographic distribution of sex.

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