Enzyme variability in large-lake *Daphnia* populations

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Allozyme studies of two primarily parthenogenetic, large-lake *Daphnia* species, *D. galeata mendotae* and *D. cucullata*, revealed high clonal diversity and levels of enzyme polymorphism similar to those found in other groups of organisms. Temporal analyses showed moderate stability of genotypes. Hardy–Weinberg deviations were detected on some dates in *D. cucullata*, most often resulting from homozygote excesses. Thus, these populations show higher genetic diversity and greater temporal stability than expected on the basis of previous studies of permanent pond populations of other cladocerans.

INTRODUCTION

Cyclical parthenogens exhibit both asexual and sexual reproduction; they therefore allow the assessment of the genetic consequences and evolutionary ramifications of switching reproductive modes. *Daphnia* populations are of interest in this respect because of their capacity for cyclical parthenogenesis and because of the link between ecological factors and the timing and intensity of sexual reproduction. The association between the characteristics of the physical environment, the relative incidence of sexual reproduction, and the genetic structure of the population, has been previously emphasized (Hebert, 1980). Most *Daphnia* populations inhabiting temporary ponds form sexual resting eggs which persist through dry periods until the proper environment reappears (some forms parthenogenetically produce resting eggs, see Hutchinson 1967, Hebert and Crease 1980). In populations inhabiting permanent water bodies, the relative constancy of the environment appears to decrease the frequency of resting egg production. In some large-lake populations, resting eggs have not been found (Hutchinson, 1967: 596).

Until recently, genetic studies of cyclically parthenogenetic *Daphnia* populations were confined to the pond species *D. magna* (Hebert, 1974a–d; Hebert and Ward, 1976; Young, 1979a, b), *D. pulex* (Berger and Sutherland, 1978; Lynch, 1983; 1984a, b), most populations of which are believed by Hebert (1983) to consist of obligately parthenogenetic individuals, and *D. carinata* (Hebert and Moran, 1980). In general, these studies have shown that permanent pond populations, when compared to temporary pond populations, tend to exhibit large fluctuations in genotypic frequencies, pronounced Hardy–Weinberg deviations, and low clonal diversity. The results suggest a relationship between the regularity of resting egg production and the genetic structure of pond populations.

Partly because of methodological problems caused by the small size of lake *Daphnia*, there are relatively few existing data on their population genetics (Mort and Jacobs, 1981; Jacobs, 1982; Mort, 1983; Wolf, 1984). Large-lake species are important not only because of their low frequency of sexual reproduction, but also because they often undergo cycles of phenotypic change. It is of interest to know the genetic structure of populations which exhibit high amounts of phenotypic variability. The present study uses allozyme data from two large-lake *Daphnia* populations to illustrate that both genotypic diversity and temporal stability of

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genotype frequencies are higher than expected on the basis of previous studies of pond species.

MATERIALS AND METHODS

Study sites and sampling

D. galeata mendotae were collected from the deepest area (13.1 m) of the steep-shored, 2.05 km² Lake Morey (Vermont, U.S.A.), by oblique tows with a 73 µm mesh plankton net. *Daphnia* were transported to the laboratory within one hour of collection, kept alive in an incubator at lake temperature, and sorted for electrophoresis within 24 hours. Reproductive analysis of the population from October 1978 to December 1981 detected, at various times, parthenogenetic females, sexual females, and males (Mort, 1983). For electrophoretic analyses, only parthenogenetic females were used; the other reproductive classes, when present, were too rare in the live samples to provide sufficient sample sizes for analysis.

To investigate the clonal structure of the *D. galeata mendotae* population, clones were established from stem females obtained from field collections made during August 1981 through May 1982. Clones were cultured in filtered lake-water at 20°C under continuous light and fed *Cryptomonas*. On each of four occasions, 12 to 89 clones were isolated. Low survivorship of clones was encountered, and was at least in part due to mortality resulting from animals caught on the surface film of the medium.

D. cucullata were collected from a 12 m deep station in the 0.82 km² Schöhsee (Plön, Holstein, F.R.G.; maximum lake depth = 30 m; mean depth = 13 m), by oblique tows with a 250 µm mesh plankton net. *Daphnia* were immediately transported to the laboratory and sorted for electrophoresis within 24 hours of collection. Although this population occasionally contains sexual females and males, only parthenogenetic females were used for electrophoresis. Clonal structure of the population was determined directly from field-caught individuals, by slicing starch gels to assay for multiple enzyme phenotypes per individual.

ELECTROPHORESIS

Daphnia galeata mendotae

Electrophoresis was carried out on 7 per cent polyacrylamide vertical slab gels (Aardvark Instru-

ments) using methods modified from Manning *et al.*, (1978). The gel consisted of 200 ml 0.1 M Tris-borate buffer (pH 8.9, 12.114 g Tris, and 0.25 g boric acid per litre water), 13.97 g Cyanogum (E-C Apparatus), 0.33 ml TEMED, and 1.66 ml 10 per cent ammonium persulphate. Individual adult females were homogenised in an Adamcewicz microhomogeniser in which 15 µl of a tris-borate (pH 8.9) + bromophenol blue + 10 per cent sucrose solution had been placed. The electrophoretic separation was carried out at 350 volts for at least 2.5 hours per application of homogenate. The gel slabs were then removed from the apparatus and incubated in the dark in stains modified from Shaw and Prasad (1970). Stain recipes are given in Mort (1983). The following enzymes were successfully stained in single individuals: glutamate oxaloacetate transaminase (GOT), phosphoglucumutase (PGM), alkaline phosphatase (ALK), alpha-esterase (EST), beta-esterase (β-EST), acid phosphatase (ACP), phosphoglucose isomerase (PGI), malic dehydrogenase (MDH), and xanthine dehydrogenase (XDH).

Daphnia cucullata

Electrophoresis was carried out on 11 per cent horizontal starch gels (DESAGA, Heidelberg) using methods modified from Wolf (1982). The gel consisted of 80 ml 0.14 M Tris-citrate buffer (pH 8.5, 16.4 g Tris, 0.4 g EDTA, and 4 g citric acid per litre water), 1.12 litres water, and 130 g starch (SIGMA). Individual adult females were homogenised in Microtiter plates with 20 µl Tris-citrate (0.01 M) and bromophenol blue. The homogenate was soaked into filter paper wicks (Whatman No. 3) and loaded onto the gels. The electrophoretic separation was carried out at 10 mA for 0.5 hours. The wicks were then removed and the separation was continued for 5.5 hours at 30 mA. The gel slab was separated into three to four slices and incubated in the dark in stains slightly modified from Wolf (1982). The major modification of the protocol is the deletion of Triton from the gel; instead, 50 µl Triton X-100/100 ml stain is added to PGI, PGM, and MDH. PGI and PGM have sufficiently different mobility so that they may be stained on the same slice. The other enzymes stained were: GOT, fumarase (FUM), lactate dehydrogenase (LDH), NADP-dependent malic dehydrogenase (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), and 6-phosphoglucuronate dehydrogenase (6-PGDH).

RESULTS

Population characteristics

In both *Daphnia* populations considered here, reproduction occurs primarily via parthenogenesis. Reproductive analysis of the *D. galeata mendotae* population from October 1978 through December 1981 showed that sexual reproduction, occurring in October through December, was low and varied from year to year. During the 3-year period of analysis, sexual females constituted a maximum of 8 per cent of the population (Mort, 1983). Similarly, in the *D. cucullata* population, sexual reproduction was detected in November and December and reached a maximum of 5 per cent during the 2-year period of 1981 and 1982 (W. Lampert, personal communication).

Cyclical phenotypic change, also known as cyclomorphosis (see Hutchinson, 1967), was exhibited by both populations. Phenotypic changes involved changes in size and shape of the head with associated changes in the length of the tailspine. The phenotypic changes follow the same pattern as water temperature; maximum expression of the characters is in late summer when temperatures are highest. In the *D. galeata mendotae* population, the head length of both juveniles and adults ranged from 8 per cent (winter) to 26 per cent (summer) of the body length, in a pattern which was repeated yearly from 1979 through 1982 (Mort, in preparation). *D. cucullata* exhibited similar, but more pronounced, phenotypic changes with a head length range of 8 per cent (winter) to 56 per cent (summer) of the body length during 1981 and 1982 (Lampert and Wolf, in preparation).

Enzyme variability

Of the nine enzyme systems examined in *D. galeata mendotae*, three (PGI, MDH, XDH) were invariant and the remaining six were polymorphic (GOT, PGM, ALK-2, EST, β -EST, ACP). Of the nine loci examined in *D. cucullata*, five were invariant (ME, FUM, LDH, G-6-PDH, 6-PGDH) and the remaining four (PGM, PGI, MDH, GOT) were polymorphic.

Enzyme phenotypes

For *D. galeata mendotae*, four polymorphic loci (GOT, PGM, ALK-2, EST-2) were used to investigate the genetic structure of the population. Segregation at these loci could not be confirmed by mating experiments because of unsuccessful attempts to induce mating in the laboratory. Thus,

the genetic basis of the enzyme patterns is inferred from laboratory-raised individuals and from previous studies (Hebert and Ward, 1972). GOT, a dimeric enzyme, exhibited two phenotypes, single-banded and triple-banded, which were identified as SS homozygotes and SF heterozygotes, respectively. PGM, a monomer, exhibited three phenotypes: two single-banded designated FF and SS homozygotes and one double-banded designated an SF heterozygote. The alpha-EST staining procedure yielded two zones of activity, EST-1 and EST-2. EST-1 activity was not found in all individuals and is not further considered. EST-2, a monomer, showed three phenotypes: two single bands designated SS and FF homozygotes and one double band designated an SF heterozygote. Four zones of alkaline phosphatase activity were detected in *D. galeata mendotae*, probably representing enzyme activity at four distinct loci. Only the second zone, ALK-2, was found in all individuals. Three enzyme phenotypes were present at ALK-2: two single-banded patterns designated SS and FF homozygotes and one double-banded pattern designated an SF heterozygote. EST-2 and ALK-2 phenotypes were stable in laboratory clones.

In *D. cucullata*, of the four polymorphic loci identified, two (PGI, PGM) were regularly used to investigate the genetic structure of the population. PGM exhibited three phenotypes: two single bands interpreted to represent SS and FF homozygotes and one double band designated an SF heterozygote. PGI, a dimeric enzyme, is polymorphic for four alleles represented by ten phenotypes: S⁺S⁺, SS, MM, FF, S⁺S⁻, S⁻M, S⁻F, SM, SF, MF. Homozygotes were single-banded; heterozygotes were triple-banded. On a few occasions, MDH activity was high enough that it could be assayed as a third locus in individual *Daphnia*. MDH, a dimeric enzyme, is polymorphic for three alleles resulting in six phenotypes: SS, MM, FF, SM, SF, MF. MDH homozygotes were single-banded; heterozygotes were triple-banded. GOT could only be assayed in exceptionally large *D. cucullata*, which were present only on a few occasions. Two alleles resulting in three phenotypes (SS, FF, SF) were detected. Homozygotes were single-banded; heterozygotes were triple-banded.

Clonal diversity

For the *D. galeata mendotae* population, clonal structure was investigated by electrophoretic analysis of 27 clones established from field collections. Analysis at four loci (GOT, PGM, ALK-2, EST-2; table 1) revealed 14 distinct genotypes.

Table 1 Clonal groups detected in the Lake Morey *D. galeata mendotae* population; based on laboratory clones originating from field collections

Genotype				Frequency	Proportion of total
GOT	PGM	ALK-2	EST-2		
SS	SS	SF	SS	1	0.037
SS	SF	SS	SS	3	0.112
SS	SF	SS	SF	7	0.259
SS	SF	SF	SS	5	0.185
SS	SF	SF	SF	2	0.074
SS	FF	SS	SS	1	0.037
SS	FF	SS	SF	1	0.037
SS	FF	SS	FF	1	0.037
SS	FF	SF	FF	1	0.037
SS	FF	FF	SF	1	0.037
SF	SF	SF	SF	1	0.037
SF	FF	SS	SS	1	0.037
SF	FF	SS	SF	1	0.037
SF	FF	FF	SF	1	0.037

Table 2 Clonal groups detected in the Schöhsee *D. cucullata* population; based on field-caught individuals

PGM	PGI	Frequency	Proportion total
SS	S ⁻ S ⁻	8	0.002
SS	SS	302	0.084
SS	MM	40	0.011
SS	FF	3	0.001
SS	S ⁻ S	124	0.035
SS	S ⁻ M	18	0.005
SS	S ⁻ F	4	0.001
SS	SM	151	0.042
SS	SF	30	0.008
SS	MF	6	0.002
SF	S ⁻ S ⁻	18	0.005
SF	SS	693	0.194
SF	MM	86	0.024
SF	FF	7	0.002
SF	S ⁻ S	273	0.076
SF	S ⁻ M	60	0.017
SF	S ⁻ F	22	0.006
SF	SM	270	0.075
SF	SF	66	0.018
SF	MF	21	0.006
FF	S ⁻ S	8	0.002
FF	SS	601	0.168
FF	MM	153	0.043
FF	FF	4	0.001
FF	S ⁻ S	215	0.060
FF	S ⁻ M	35	0.010
FF	S ⁻ F	11	0.003
FF	SM	247	0.069
FF	SF	92	0.026
FF	MF	14	0.004

Since 54 genotypic combinations are possible and since most genotypes were rare, analysis of a larger number of clones would probably increase the number of genotypes detected. Because analysis at more loci would also be likely to increase the number of genotypes detected, this technique identifies clonal groups which can themselves contain a large number of distinct genotypes. Thus, the present analysis suggests that the population contains a large number of distinct genotypes which, because reproduction is primarily parthenogenetic, can be taken as evidence that the population contains a large number of clones.

D. cucullata individuals were routinely characterised at both PGM and PGI. During 1982, all of the 30 possible PGI-PGM genotypes were detected (table 2). Linkage relationships between the loci were determined with an EM (Expectation-Maximization) algorithm (C. Strobeck, personal communication; modified from Cheliak *et al.*, 1983). Significant linkage disequilibrium was detected on one date: 15.6.82 ($\chi^2 = 10.78$, $p < 0.05$). Thus, little association exists between the two loci. In May 1983, *D. cucullata* individuals were also characterised at MDH. Using the 3-locus genotypes (PGI-PGM-MDH), 60 of the 180 possible combinations were detected. The missing combinations were those containing rare alleles. Thus, as in the *D. galeata mendotae* population, the *D. cucullata* population consists of a large number of coexisting clones.

Temporal analysis of genotypes

Analysis of the *D. galeata mendotae* population at the GOT locus showed that the genotypes remained fairly constant over time (fig. 1) with no significant temporal heterogeneity ($G = 13.23$, $p = 0.5$). The GOT-FF genotype was not detected, probably because of its low expected frequency (table 3). Genotypic frequencies showed no significant deviations from Hardy-Weinberg proportions as determined by χ^2 tests performed for each sampling date. With an allele near fixation, however, it is difficult to detect such deviations. Calculation of $H = -(\sum H_{\text{exp}} - \sum H_{\text{obs}}) / \sum H_{\text{exp}}$, where $\sum H_{\text{exp}}$ is the sum of expected heterozygote frequencies and $\sum H_{\text{obs}}$ is the sum of observed heterozygote frequencies, as a measure of deviations from Hardy-Weinberg proportions reveals a permanent heterozygote excess (H always positive). Also a χ^2 test on the pooled data show significant deviations from Hardy-Weinberg proportions ($\chi^2 = 15.7$, $p < 0.001$).

Figure 1 Temporal analysis of GOT genotypes in *D. galeata mendotae*, Lake Morey.

Because of scoring difficulties, data at the ALK-2 locus is limited to 1981. At ALK-2, genotypes remained stable (fig. 2) until the October sample when an increase in both SF and FF genotypes occurred (table 4), resulting in significant temporal heterogeneity ($G = 36.72$, $p < 0.001$). Genotypic frequencies at ALK-2 showed no significant deviations from Hardy-Weinberg proportions which, as at the GOT locus, may have been difficult to detect because of the high frequency of the S allele.

With some exceptions, *D. cucullata* PGM genotypes remained fairly constant from one

sampling date to the next but show significant heterogeneity when the entire 8-month sampling period is considered ($G = 93.19$, $p < 0.001$; fig. 3). The FF genotype increased in frequency over this period (table 5). Of the 26 sampling dates, five showed significant deviations from Hardy-Weinberg proportions due to homozygote excesses (table 5). Even when deviations are not statistically significant, the—with two exceptions—constantly negative value of H suggests homozygote excess.

At PGI, *D. cucullata* genotypes were moderately stable over time (fig. 4) but also showed

Table 3 GOT genotype and allele frequencies and relative deviations of heterozygote frequencies from Hardy-Weinberg expectations for the *D. cucullata* population

Collection date	N	Genotype frequencies			Allele frequencies		H
		SS	SF	FF	S	F	
13.6.80	47	0.720	0.280	0.000	0.860	0.140	+0.163
25.9.80	20	0.750	0.250	0.000	0.880	0.120	+0.184
1.6.81	115	0.704	0.296	0.000	0.852	0.148	+0.174
7.7.81	76	0.763	0.237	0.000	0.882	0.118	+0.139
31.7.81	93	0.817	0.183	0.000	0.909	0.091	+0.106
14.8.81	114	0.781	0.219	0.000	0.891	0.109	+0.127
28.8.81	109	0.817	0.183	0.000	0.909	0.091	+0.106
14.9.81	107	0.766	0.234	0.000	0.883	0.117	+0.133
1.10.81	29	0.793	0.207	0.000	0.897	0.103	+0.120
15.10.81	75	0.773	0.227	0.000	0.886	0.114	+0.124
13.11.81	102	0.873	0.127	0.000	0.937	0.063	+0.076
1.12.82	128	0.844	0.156	0.000	0.922	0.078	+0.085
27.5.82	66	0.712	0.288	0.000	0.856	0.144	+0.168

Figure 2 Temporal analysis of ALK-2 genotypes in *D. galeata mendotae*, Lake Morey.

significant temporal heterogeneity ($G = 234.30$, $p < 0.001$) due to an increase in frequency of the SS genotype (table 6). Deviations from Hardy-Weinberg proportions were more common at this locus; significant deviations were detected on 15 of the 26 sampling dates (table 6). χ^2 tests for Hardy-Weinberg deviations were performed on six classes which included three composite classes containing rare genotypes: 1) S^-S^- , FF , S^-F ; 2) S^-S , SF ; 3) S^-M , MF . On dates when significant deviations were detected they were due to an excess of one or both of the common homozygotes (SS, MM) usually accompanied by a smaller excess of at least one of the heterozygotes or heterozygote classes (no heterozygote excesses were present on 24.8 and 19.10). This small heterozygote excess was not sufficient to change the overall picture of homozygote excess as shown by a negative value of H . However, on one occasion (11.5) there were significant deviations from Hardy-Weinberg proportions due to heterozygote excess. In general, *D. cucullata* showed a tendency towards homozygote excesses at both loci (value of H negative).

Table 4 ALK-2 genotype and allele frequencies and relative deviations of heterozygote frequencies from Hardy-Weinberg expectations for the *D. galeata mendotae* population

Collection date	N	Genotype frequencies			Allele frequencies		H
		SS	SF	FF	S	F	
19.6.81	62	0.726	0.242	0.032	0.847	0.153	-0.066
3.7.82	16	0.750	0.188	0.062	0.844	0.156	-0.286
31.7.81	87	0.724	0.276	0.000	0.862	0.138	+0.160
14.8.81	85	0.788	0.200	0.012	0.888	0.112	+0.005
28.8.81	95	0.853	0.147	0.000	0.927	0.073	+0.086
15.10.81	53	0.453	0.472	0.075	0.689	0.311	+0.101

Figure 3 Temporal analysis of PGM genotypes in *D. cucullata*, Schöhsee.

Table 5 PGM genotype and allele frequencies and relative deviations of heterozygote frequencies from Hardy-Weinberg expectations for the *D. cucullata* population

Collection date	N	Genotype frequencies			Allele frequencies		H
		SS	SF	FF	S	F	
16.3.82	158†	0.259	0.342	0.399	0.430	0.570	-0.302
23.3.82	95*	0.221	0.368	0.411	0.405	0.595	-0.236
30.3.82	95	0.221	0.453	0.326	0.447	0.553	-0.084
6.4.82	96	0.219	0.406	0.375	0.422	0.578	-0.168
13.4.82	72	0.208	0.417	0.375	0.417	0.583	-0.142
20.4.82	96	0.208	0.385	0.407	0.401	0.599	-0.199
26.4.82	131	0.199	0.480	0.321	0.439	0.561	-0.025
4.5.82	87	0.241	0.414	0.345	0.448	0.552	-0.163
11.5.82	87	0.264	0.472	0.264	0.500	0.500	-0.056
17.5.82	97	0.155	0.474	0.371	0.392	0.608	-0.006
25.5.82	156	0.276	0.385	0.339	0.468	0.532	-0.227
1.6.82	135	0.237	0.356	0.407	0.415	0.585	-0.267
8.6.82	93	0.226	0.430	0.344	0.441	0.559	-0.128
15.6.82	97*	0.196	0.330	0.474	0.361	0.639	-0.285
29.6.82	188*	0.197	0.399	0.404	0.396	0.604	-0.166
13.7.82	191	0.199	0.476	0.325	0.437	0.563	-0.033
27.7.82	186	0.156	0.462	0.382	0.387	0.613	-0.026
10.8.82	187	0.198	0.438	0.364	0.417	0.583	-0.099
24.8.82	188	0.213	0.457	0.330	0.442	0.558	-0.074
7.9.82	185	0.108	0.497	0.395	0.357	0.643	+0.083
20.9.82	179	0.229	0.391	0.380	0.425	0.575	-0.200
5.10.82	187	0.134	0.438	0.428	0.353	0.647	-0.041
19.10.82	100*	0.180	0.320	0.500	0.340	0.660	-0.287
2.11.82	180	0.150	0.494	0.356	0.397	0.603	+0.032
16.11.82	148	0.108	0.412	0.480	0.314	0.686	-0.044
29.11.82	169	0.112	0.391	0.497	0.308	0.692	-0.083

* $P < 0.05$.

† $p < 0.01$; χ^2 test for Hardy-Weinberg deviations.

Figure 4 Temporal analysis of PGI genotypes in *D. cucullata*, Schöhsee.

Table 6 PGI genotype and allele frequencies and relative deviations of heterozygote frequencies from Hardy-Weinberg expectations for the *D. cucullata* population

Collection Date	N	Genotype frequencies										Allele frequencies				
		S ⁻ S ⁻	SS	MM	FF	S ⁻ S	S ⁻ M	S ⁻ F	SM	SF	MF	S ⁻	S	M	F	H
16.3.82	158*	0.013	0.387	0.076	0.044	0.171	0.044	0.006	0.209	0.044	0.006	0.123	0.598	0.206	0.073	-0.172
23.3.82	95	0.032	0.316	0.074	0.000	0.147	0.084	0.000	0.284	0.063	0.000	0.147	0.563	0.258	0.032	-0.027
30.3.82	95	0.011	0.431	0.063	0.000	0.116	0.042	0.000	0.242	0.084	0.011	0.089	0.653	0.211	0.047	-0.046
6.4.82	96	0.021	0.428	0.073	0.010	0.125	0.021	0.010	0.260	0.042	0.010	0.099	0.640	0.219	0.042	-0.116
13.4.82	72	0.014	0.375	0.028	0.014	0.236	0.083	0.000	0.194	0.042	0.014	0.174	0.610	0.174	0.042	+0.006
20.4.82	96*	0.010	0.458	0.115	0.000	0.146	0.021	0.000	0.104	0.125	0.021	0.094	0.645	0.188	0.073	-0.220
26.4.82	131*	0.015	0.359	0.099	0.000	0.160	0.092	0.015	0.191	0.046	0.023	0.149	0.557	0.252	0.042	-0.125
4.5.82	87	0.012	0.355	0.058	0.000	0.184	0.058	0.023	0.195	0.092	0.023	0.144	0.592	0.195	0.069	-0.019
11.5.82	87*	0.000	0.322	0.069	0.000	0.264	0.023	0.035	0.230	0.057	0.000	0.161	0.598	0.195	0.046	+0.057
17.5.82	97	0.000	0.454	0.052	0.000	0.165	0.041	0.031	0.206	0.041	0.010	0.119	0.660	0.180	0.041	-0.043
25.5.82	156	0.000	0.442	0.071	0.000	0.199	0.026	0.019	0.186	0.051	0.006	0.122	0.660	0.180	0.038	-0.056
1.6.82	135	0.007	0.401	0.044	0.000	0.193	0.030	0.037	0.207	0.059	0.022	0.137	0.630	0.174	0.059	-0.005
8.6.82	93	0.000	0.322	0.022	0.000	0.226	0.032	0.043	0.247	0.097	0.011	0.151	0.607	0.167	0.075	+0.140
15.6.82	97	0.010	0.350	0.052	0.010	0.227	0.052	0.000	0.237	0.052	0.010	0.149	0.609	0.201	0.041	+0.023
29.6.82	188†	0.021	0.379	0.138	0.016	0.154	0.032	0.005	0.170	0.064	0.021	0.117	0.572	0.250	0.061	-0.248
13.7.82	191†	0.011	0.402	0.089	0.000	0.189	0.052	0.011	0.157	0.047	0.042	0.136	0.599	0.215	0.050	-0.132
27.7.82	186†	0.005	0.447	0.102	0.005	0.199	0.011	0.000	0.172	0.054	0.005	0.110	0.659	0.196	0.035	-0.142
10.8.82	187	0.011	0.530	0.037	0.000	0.166	0.021	0.000	0.187	0.032	0.016	0.104	0.722	0.150	0.024	-0.051
24.8.82	188*	0.011	0.521	0.080	0.000	0.133	0.032	0.005	0.181	0.037	0.000	0.096	0.697	0.186	0.021	-0.068
7.9.82	185*	0.000	0.497	0.054	0.000	0.168	0.022	0.022	0.162	0.070	0.005	0.105	0.697	0.149	0.049	-0.108
20.9.82	179*	0.006	0.468	0.050	0.000	0.212	0.017	0.006	0.207	0.028	0.006	0.123	0.692	0.165	0.020	-0.005
5.10.82	187*	0.011	0.541	0.069	0.000	0.187	0.016	0.005	0.144	0.016	0.011	0.115	0.714	0.155	0.016	-0.163
19.10.82	100*	0.010	0.530	0.220	0.000	0.080	0.000	0.010	0.100	0.030	0.020	0.055	0.635	0.280	0.030	-0.533
2.11.82	180*	0.006	0.488	0.100	0.000	0.161	0.017	0.006	0.161	0.061	0.000	0.097	0.681	0.189	0.033	-0.171
16.11.82	148*	0.014	0.491	0.095	0.000	0.169	0.020	0.007	0.149	0.041	0.014	0.112	0.672	0.186	0.030	-0.201
29.11.82	169*	0.006	0.568	0.065	0.000	0.101	0.006	0.000	0.195	0.059	0.000	0.059	0.745	0.166	0.030	-0.126

* $p < 0.05$.† $p < 0.01$; χ^2 tests for Hardy-Weinberg.

DISCUSSION

The level of enzyme variability in both *D. galeata mendotae* (66 per cent, based on nine loci) and *D. cucullata* (44 per cent, based on nine loci) is higher than the 11 per cent polymorphism reported by Hebert (1980) for *D. magna* (based on 15 loci) and *D. carinata* (based on 17 loci, Hebert and Moran, 1980). Since the present estimates of polymorphism are based on fewer loci, it can be argued that the addition of more loci would decrease the estimates. If six additional loci had been examined and found to be monomorphic, the levels of polymorphism would remain high at 40 per cent for *D. galeata mendotae* and 26 per cent for *D. cucullata*. In addition, more recent studies on *D. pulex* report 47 per cent polymorphism (based on 17 loci, Berger and Sutherland, 1978) and 67 per cent polymorphism (based on 15 loci, Lynch 1984a). On the basis of the low levels of polymorphism and the differences in the clonal composition of different populations of *D. magna* and *D. carinata* Hebert (1980) suggested that *Daphnia* populations

were prone to founder effects followed by inbreeding because of the presence of only a small part of the genetic variability of the species in any single population. This conclusion does not seem to be extendable to populations of other *Daphnia* species, which exhibit levels of polymorphism similar to the 30 per cent to 50 per cent detected in other organisms (Lewontin, 1974). Although we cannot estimate the proportion of the total species variability present in the two populations examined, studies of several large-lake populations of *D. galeata* give little indication of differentiation between populations (Mort and Wolf, in preparation).

Both the *D. galeata mendotae* and *D. cucullata* populations consist of a large number of multi-locus genotypes. In *D. galeata mendotae*, 50 per cent of the possible number of 4-locus genotypes were detected; a larger sample size would very likely increase this proportion. In *D. cucullata*, all of the 2-locus (PGI-PGM) combinations were found. Because reproduction is primarily parthenogenetic, these genotypes can be taken to represent

distinct clonal groups which themselves contain a large number of clones. Thus, the clonal diversity detected in the present study approaches that which has been detected in temporary populations of other *Daphnia* where the entire population undergoes sexual reproduction (Young, 1979b; Lynch, 1984a). Hebert (1984) and Lynch (1983; 1984a) have suggested that clonal diversity in the primarily parthenogenetic large-lake populations should be low due to the erosion of genotypic diversity via long-term selection. If long-term erosion of clonal diversity is occurring in the populations considered here, then it is slight, and the low amount of sexual reproduction is apparently sufficient to re-establish genotypic diversity. Genetic diversity could also be augmented by hatching of sexual resting eggs produced during previous seasons. Resting eggs deposited in the lake sediments can persist for several years before hatching (Herzig, 1984). The resting eggs may provide a reservoir from which genetic diversity could be replenished, especially after periods of harsh selection or population bottle-necks.

In *D. galeata mendotae*, temporal stability of genotypes was observed at the GOT locus but not at the ALK-2 locus. The large changes at the ALK-2 locus in the October sample coincided with the onset of sexual reproduction. Since sexual females were not used for electrophoresis, the changes in genotypic frequencies may reflect differential expression of sexual reproduction among genotypes, as has been noted in *D. pulex* (Lynch, 1983), although the changes are too large to be explained only by this phenomenon. In the *D. cucullata* population, significant temporal heterogeneity was observed at both PGM and PGI. In both cases, the temporal heterogeneity was produced by a rise in frequency of a common homozygote, suggesting a shift in the genetic structure of the population.

Thus, significant temporal heterogeneity of genotypes was observed in both the *D. galeata mendotae* and the *D. cucullata* populations. The temporal fluctuations, however, are not as pronounced as those reported by Hebert and Ward (1976) for permanent *D. magna* populations. The present study provides evidence for relative stability of genotypes in lake populations when compared to permanent pond populations. Jacobs (1982), however, reports larger shifts in genotypic frequencies from summer to winter in *D. cucullata* and *D. hyalina* (another lake species). Study of more populations is needed before a general statement can be made about patterns of genotypic fluctuations in lakes.

Many studies of permanent *Daphnia* populations have revealed significant deviations from Hardy-Weinberg proportions mostly due to heterozygote excesses (Hebert, 1983). Hebert (1983) has suggested that such findings signify heterosis and should be expected in permanent populations where prolonged parthenogenesis allows the time for culling of genotypes by natural selection. In the present study, the *D. galeata mendotae* population exhibited no deviations from Hardy-Weinberg proportions at two loci on any single sampling date; only after pooling all samples was there evidence for heterozygote excess at the GOT-locus. The *D. cucullata* population exhibited, at various times, Hardy-Weinberg deviations at both PGI and PGM. At the PGM locus, deviations were due to homozygote excesses. At the PGI locus, homozygote excesses were more common than heterozygote excesses but both types were observed. Thus, in the present study, there is a lack of compelling evidence for the heterosis thought to be operating in pond populations (Hebert, 1983), although more populations must be examined before general statements can be made.

Experimental manipulation of natural populations indicates that *D. galeata mendotae* genotypes respond differently to strong environmental perturbations (Mort and Jacobs, 1981). The present study, however, suggests a stable genotypic assemblage with only weak selection acting among clones under natural levels of environmental change. If lake clones are responding differently to environmental changes, then the effect may be slight shifts in clonal abundances rather than the clonal replacement seen in pond populations (Lynch, 1983). The results of the present study seem more consistent with Lynch's hypothesis (1983) that permanent populations should consist of generalist genotypes selected for similar phenotypic responses to a seasonal environment. The regularity of the phenotypic cycles exhibited by lake populations may be an indication that different genotypes have been selected for similarity in average phenotypic responses.

Acknowledgements We thank Jürgen Jacobs and an anonymous reviewer for valuable comments on earlier drafts of this paper, Eva Geißler for technical assistance, and Winfried Lampert and the members of the Abteilung Ökophysiologie for general support and assistance. A portion of the data is contained in a Ph.D. thesis submitted by M.A.M. to Dartmouth College. Financial support was provided by Dartmouth College, Sigma-Xi, and the Max-Planck-Gesellschaft.

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