ENZYME VARIABILITY IN NATURAL POPULATIONS OF *DAPHNIA MAGNA* III. GENOTYPIC FREQUENCIES IN INTERMITTENT POPULATIONS

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ABSTRACT

In temporary habitats populations of the cyclical parthenogen, *Daphnia magna*, are re-established each year from sexual eggs and reproduce parthenogenetically for two or at most three generations. The genetic effects of this breeding system have been investigated by analyzing allozyme frequencies in nineteen intermittent populations.—Genotypic frequencies at polymorphic loci were ordinarily found to be in good agreement with Hardy-Weinberg proportions and disequilibria between loci were not observed. Although significant changes in gene frequencies were observed both during and between successive cycles, there was no evidence of the marked instability of genotype frequencies characteristic of permanent populations. The recombinational degradation of genotypes at the end of each annual cycle in temporary habitats effectively prevents the genotypic structuring which develops when continued parthenogenesis is possible.

THE annual cycle of population growth was similar in all of the intermittent populations studied. The populations normally reappeared between December and February—the exact time depending largely upon when the habitats filled with water. Emergence of sexual eggs continued over a period of several weeks, with only female individuals being produced. After this initial period of re-establishment parthenogenetic multiplication of the population became important. By mid-May or June male individuals were produced parthenogenetically, presumably in response to increasingly crowded conditions, although photoperiod may also have been important (BANTA and BROWN 1929; STROSS and HILL 1965). At this time or a little later females in the population produced sexual eggs which needed to be fertilized in order to develop. The sexual period was normally terminated when the pond dried up. The populations were refounded in the following year solely from these sexual eggs, as parthenogenetic individuals were unable to survive the period of desiccation.

A more detailed introduction into the aims of the present study as well as a section detailing MATERIAL AND METHODS have been included in the companion paper on permanent populations.

RESULTS

Genotypic frequencies: Eleven temporary populations were analyzed on only a

TABLE 1

Genotypic frequencies in intermittent populations†

Location and sample date	*n*	*SS*	*SM*	*MM*	*MF*	*FF*	*SF*
Goose Hall-1	122			.22	.53	.25	
4/2/72	*120*			*1.00*			
Goose Hall-2	95			.26	.54	.20	
4/2/72	*120*			*.98*	*.02*		
Goose Hall-3	165			.07	.46	.47	
4/2/72	*96*			*.88*	*.12*		
Oakington	144			.20	.42	.38	
4/10/72	*143*			*.19*	*.50*	*.31*	
Upware Farm	144			.29	.50	.21	
4/16/72	*144*	*.04*	*.11*	*.06*	*.32*	*.24*	*.23*
Before Wickem	120			.27	.54	.19	
4/16/72	*140*	*.57*	*.12*	*.01*	*.07*	*.03*	*.20*
Stow Longa-1	96			.78	.19	.03	
4/21/72	*95*			*.73*	*.23*	*.04*	
Upthorpe	120	.01	.06	.91	.02		
4/21/72	*96*			*1.00*			
Winston Moat	96			.99	.01		
5/6/72	*120*			*1.00*			
Wooley	96			1.00			
4/21/72	*120*			*.55*	*.34*	*.11*	
Upware-2	217**	.01	.15	.50	.33	.01	
7/6/70	*119**	*.01*	*.08*	*.15*	*.36*	*.16*	*.24*

* $p < .02$
** $p < .001$
† Roman : *MDH*
Italic :*EST-1*

single occasion. Genotypic frequencies at the polymorphic loci in these populations have been compared with Hardy-Weinberg (H.-W.) proportions.

Genotypic frequencies among populations polymorphic for esterase-1 (*EST-1*) and malate dehydrogenase (*MDH*) were generally in good agreement with H.-W. expectations, but in the population at Upware-2 highly significant deviations were observed at both loci (Table 1). At the *EST-1* locus *SF* heterozygotes were much more frequent and FF homozygotes much less frequent than expected, while at the *MDH* locus there was an excess of *MF* heterozygotes and a deficiency of the *FF* and *SF* genotypes. The small deviations from H.-W. proportions in the other populations were not the result of a consistent deficiency of any one genotypic class.

Only the population at Stow Longa-1 was polymorphic for electrophoretic variants of tetrazolium oxidase. When 96 individuals from the population were analyzed on April 21, 1972, the frequencies of the *SS* and *FF* homozygotes were .21 and .26, respectively, while the frequency of the SF heterozygote was .53. These frequencies were not significantly different from H.-W. expectations ($\chi_1^2 = 1.60$, $p > .20$).

Temporal analysis of genotypic frequencies: Repeated analyses of genotypic

TABLE 2

Genotypic frequencies at Bourn and Landbeach

Date	*n*	*MDH* *SS*	*SM*	*MM*	*MF*	*FF*	*SF*	*n*	*EST-1* *SS*	*SM*	*MM*	*MF*	*FF*	*SF*
						BOURN								
4/21/71	114	.03	.07	.13	.32	.28	.17	48		.19	.60	.15		.06
5/20/71	96	.01	.13	.12	.38	.27	.09	24		.21	.33	.29		.17
2/25/72	24		.04	.12	.58	.08	.18	24		.04	.42	.46	.04	.04
4/18/72	144	.01	.09	.16	.37	.26	.11	144	.01	.19	.50	.22	.03	.05
5/19/72	144	.02	.08	.12	.42	.24	.12	120	.01	.16	.47	.28	.02	.06
						LANDBEACH								
4/27/70	315	.02	.31	.67										
5/18/70	144	.03	.28	.69										
1/11/71	16	.19	.25	.56				8	.12		.88			
2/ 2/71	132	.04	.33	.63				128	.04	.45	.50	.01		
3/ 3/71	164	.06	.33	.61				164	.08	.45	.45	.02		
4/19/71	24		.46	.54										
3/31/72	144	.04	.26	.69	.01			144	.09	.39	.50	.01	.01	
5/11/72	144	.05	.29	.66				144	.06	.42	.50	.02		

frequencies were made on eight temporary populations to ascertain if changes in frequencies occurred within the populations either during a single year or between successive years. Genotypic frequencies have been compared to H.-W. proportions only when the frequencies were based on an analysis of 48 or more individuals.

Bourn: The population at Bourn was polymorphic for three alleles of both *MDH* and *EST-1*. (Table 2). Gene frequencies at the *MDH* locus were similar in the 1971 and 1972 samples and in all cases genotypic frequencies were close to H.-W. proportions. Gene frequencies at the *EST-1* locus were apparently also quite similar in 1971 and 1972, although the 1971 samples were too small to allow for critical comparison. Again genotypic frequencies were close to H.-W. proportions.

Landbeach: The population at Landbeach was polymorphic for the *S* and *F* alleles of MDH (Table 2). Gene frequencies in 1970 were similar to those in 1971 and 1972 and in each of the samples genotypic frequencies were similar to H.-W. expectations. In 1971 the Landbeach pond dried up before the population began to reproduce sexually. As a result, the 1972 population must have been founded from ephippia which had remained unhatched for at least two years.

The Landbeach population was segregating for three alleles of *EST-1*, although the frequency of the *M* allele was very low (Table 2). Gene frequencies were similar in 1971 and 1972 and genotypic frequencies were in close agreement with H.-W. proportions.

Each individual in the 1971 and 1972 March samples was analyzed for both *MDH* and *EST-1*. Frequencies were not significantly different from those expected assuming there was a random association of *EST-1* and *MDH* genotypes (χ_4^2 (1971) $= 6.98$, $p > .10$; χ_4^2 (1972) $= 8.87$, $p > .05$).

TABLE 3

EST-1 *frequencies at Kimbolton*

Date	*n*	Genotypic frequencies *SS*	*SF*	*FF*
7/22/70	96	.23	.48	.29
5/31/71	96	.30	.48	.22
7/ 9/71	96	.26	.51	.23
5/29/72	120	.24	.51	.25

Kimbolton: A single analysis in 1970 revealed that the Kimbolton population was polymorphic for the *S* and *F* alleles of *EST-1* with a gene frequency close to .50 (Table 3). No changes in gene frequency were detected in the samples analyzed in 1971 and 1972. Genotypic frequencies were close to H.-W. proportions in all the samples.

Longstowe Field-1: The Longstowe Field population was polymorphic for the S and *M* alleles of *MDH* (Table 4). Genotypic frequencies in the population were stable throughout 1971 ($\chi_8^2 = 6.22$, $p > .50$), and in 1972 ($\chi_2^2 = 0.72$, $p > .50$), but large differences in genotypic frequencies were apparent between the years ($\chi_2^2 = 13.95$, $p < .001$). Despite the reduction in frequency of the *M* allele genotypic frequencies were in close agreement with H.-W. expectations in all samples.

Longstowe Moat: The population at Longstowe Moat was polymorphic for three alleles of *MDH* (Table 4). Genotypic frequencies in the three 1971 samples were similar, as were genotypic frequencies in three 1972 samples. However, the

TABLE 4

MDH *genotypic frequencies*

Date	*n*	LONGSTOWE FIELD-1 *SS*	*SM*	*MM*
5/ 4/71	100	.12	.46	.42
6/14/71	96	.12	.40	.48
8/26/71	96	.12	.39	.49
9/28/71	96	.20	.41	.39
10/20/71	32	.13	.44	.43
5/19/72	94	.22	.51	.27
6/14/72	120	.20	.48	.32

Date	*n*	LONGSTOWE MOAT *SS*	*SM*	*MM*	*MF*
5/ 4/71	96	.01	.14	.80	.05
6/14/71	96		.15	.81	.04
8/26/71	19	.05	.16	.74	.05
2/25/72	96		.04	.93	.03
3/27/72	120		.06	.90	.04
5/19/72	120		.05	.91	.04

TABLE 5

Genotypic frequencies at Toft and Girton

Date	n	MDH MM	MDH MF	MDH FF	n	EST-1 SS	EST-1 SM	EST-1 MM	EST-1 MF	EST-1 FF	EST-1 SF
					TOFT						
3/31/71	96	.84	.16		48		.02	.19	.50	.23	.06
4/20/71	32	.91	.06	.03	48			.17	.52	.27	.04
7/19/71	96	.85	.14	.01	96		.03	.16	.52	.23	.06
3/27/72	72	.90	.10		72		.03	.31	.39	.24	.03
5/19/72	120	.88	.11	.01	120		.02	.21	.49	.24	.04
					GIRTON						
6/ 5/70	24	.56	.40	.04							
3/15/71	8	.63	.37								
4/ 2/71	72	.60	.32	.08	72	.11	.45	.44			
5/ 3/71	148	.54	.40	.06							
6/ 2/71	96	.58	.34	.08	89	.10	.48	.42			
4/12/72	143	.65	.33	.02	144	.05	.35	.60			
5/13/72	144	.58	.36	.06	120	.09	.36	.55			

frequency of the *SM* genotype in the 1972 samples was significantly less than its frequency in the 1971 samples ($\chi_1^2 = 13.8$, $p < .001$). Genotypic frequencies were in close agreement with H.-W. proportions in all of the samples.

Toft: The Toft population was polymorphic for two alleles of MDH and three esterase alleles (Table 5). Gene frequencies were similar in 1971 and 1972 at both loci, while genotypic frequencies were in good agreement with H.-W. expectations in all samples.

Girton: The population at Girton was polymorphic for the *M* and *F* alleles of *MDH* (Table 5). Gene frequencies in the small 1970 sample were similar to those observed in 1971 and 1972. No significant deviations from H.-W. proportions were observed in any of the samples.

The Girton population was also segregating for the *S* and *M* alleles of *EST-1* (Table 5). Genotypic frequencies in the two 1971 samples were similar ($\chi_2^2 = 0.16$, $p > .90$), as were genotypic frequencies in the 1972 samples ($\chi_2^2 = 2.13$, $p > .30$). However, the genotypic frequencies in the 1971 samples were markedly different from those in 1972 ($\chi_2^2 = 9.10$, $p < .02$) as a result of the decreased frequency of the *S* allele in 1972. Genotypic frequencies were in close agreement with H.-W. expectations in all the samples.

Upware-1: The Upware-1 population was polymorphic for the *M* and *F* alleles of *EST-1* (Table 6). Genotypic frequencies in the three 1970 samples were not significantly heterogenous ($\chi_4^2 = 2.61$, $p > .50$), although there was some indication that the frequency of the *FF* homozygote increased during the course of the samples. Genotypic frequencies early in 1971 were similar to those in 1970 ($\chi_2^2 = 0.83$, $p > .50$), but during the course of the year marked changes in genotypic frequencies were observed ($\chi_8^2 = 15.89$, $p < .05$). During the 1972 season genotypic frequencies remained stable ($\chi_{10}^2 = 7.44$, $p > .50$).

TABLE 6

EST-1 *frequencies at Upware-1*

Date	*n*	Genotypic frequencies *MM*	*MF*	*FF*
6/23/70	144	.32	.52	.16
7/ 6/70	96	.28	.52	.20
7/14/70	96	.32	.45	.23
2/ 9/71	68	.29	.49	.22
3/15/71	146	.28	.50	.22
4/13/71	144	.32	.55	.13
5/24/71	144	.31	.55	.14
6/29/71	141	.26	.45	.29
4/ 1/72	144	.39	.46	.15
4/16/72	120	.32	.51	.17
5/ 5/72	144	.42	.43	.15
5/26/72	120	.30	.53	.17
6/ 9/72	144	.34	.46	.20
6/23/72	143	.32	.51	.17

DISCUSSION

Genotypic frequencies in intermittent populations were invariably in good agreement with Hardy-Weinberg expectations when analyzed within a few weeks of re-establishment. Such agreement indicates that populations are founded each year from a fairly large number of individuals. Furthermore, since the sexual eggs which emerge at the beginning of one cycle are likely to be derived from a number of temporally isolated populations, agreement with Hardy-Weinberg proportions suggests that gene frequencies are relatively stable from year to year. Temporal analysis of populations confirmed this supposition. No significant changes in genotypic frequencies were observed in the Landbeach, Kimbolton and Toft populations. In other populations, however, there was evidence of minor shifts in allele frequencies. At Upware-1 and Girton gene frequencies at the *EST-1* locus were different in 1971 and 1972, while at Longstowe Field-1 and at Longstowe Moat changes in the frequencies of MDH alleles were observed. These changes may have been the result of stochastic processes, but at Girton there was some evidence against this view, for gene frequencies did not change at the MDH locus despite a change at the *EST-1* locus.

During parthenogenetic reproduction the dominance and interaction components of the total variance in fitness can be exploited in addition to the additive component (FISHER 1930). Due to the ameiotic nature of parthenogenesis in Daphnia (MORTIMER 1936; HEBERT and WARD 1972) differences in genotypic fitnesses resulting from epistasis or heterosis should be reflected in disequilibria between loci and deviations from Hardy-Weinberg proportions. No evidence of epistasis was observed between the *EST-1* and the *MDH* loci in the Landbeach population in either 1971 or 1972. In the Upware-2 population highly significant deviations from Hardy-Weinberg proportions were observed at both the *MDH* and *EST-1* loci after several generations of parthenogenesis. This observation

was clearly exceptional, however, as similar differences were not observed in any of the other populations. The absence of Hardy-Weinberg disturbances might be taken as an indication that genotypic frequencies were stable. This was not so in the Upware-1 population. During the 1971 cycle relatively pronounced changes in genotypic frequencies were observed, but the direction of change altered during the course of the season and as a result genotypic frequencies did not deviate significantly from Hardy-Weinberg proportions in any single sample.

The results obtained in the present study are strikingly different from those obtained in a study of permanent populations of *D. magna*. The marked instability of genotypic frequencies, the large deviations from Hardy-Weinberg expectations, and the pronounced disequilibria between loci characteristic of permanent populations were either not observed or were exceptional in intermittent populations. The present study demonstrates, then, the striking effect which environmental factors can have on the genetic effects of a breeding system. Apparently the adaptive differentiation which cyclical parthenogenesis makes possible in permanent habitats is largely prevented by the recurrent bouts of recombination imposed on intermittent populations.

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LITERATURE CITED

BANTA, A. M. and L. A. BROWN, 1929 Control of sex in cladocera I. Crowding the mothers as a means of controlling male production. Physiol. Zool. **2**: 80–92.

FISHER, R. A., 1930 *The genetical theory of natural selection.* Clarendon Press, Oxford.

HEBERT, P. D. N. and R. D. WARD, 1972 Inheritance during parthenogenesis in *Daphnia magna.* Genetics **71**: 639–642.

MORTIMER, C. H., 1936 Experimentelle und cytologische Untersuchungen uber den Generationwechelsel der Cladoceren. Zool Jb. Physiol. **56**: 323–388.

STROSS, R. G. and J. C. HILL, 1965 Diapause induction in Daphnia requires two stimuli. Science **150**: 1462–1464.

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