

ENZYME POLYMORPHISM AND CYCLIC PARTHENOGENESIS
IN *DAPHNIA MAGNA*. II. HETEROSIS FOLLOWING
SEXUAL REPRODUCTION

J. P. W. YOUNG*

Department of Genetics, University of Cambridge, England

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ABSTRACT

Cyclical parthenogenesis exaggerates the force of selection relative to recombination and will therefore enhance interlocus effects. Observations of electrophoretic variation in a natural population of *Daphnia magna* Straus (Crustacea: Cladocera) are interpreted in this light. Sexual reproduction led to Hardy-Weinberg equilibrium, but heterozygote excesses rapidly developed at each of three observed loci during subsequent parthenogenesis. Homozygote fecundity was often lower than that of heterozygotes; this may have been the cause of some of the observed frequency changes. The superior fitness of the enzyme heterozygotes does not imply that selection was necessarily acting on the enzyme loci themselves, since apparent heterosis is the expected result of linkage disequilibrium.

D*APHNIA magna* is an animal in which selection is greatly magnified relative to recombination. The result is that linkage disequilibria between loci will arise readily and decay slowly. Effects that depend on such disequilibria, including "associative overdominance" (FRYDENBERG 1963) and "hitchhiking" (MAYNARD SMITH and HAIGH 1974), should be enhanced. The cause of this distorted relationship between genetic parameters is *Daphnia's* life history, which is sometimes called "cyclical parthenogenesis." Cladocerans are small freshwater crustaceans whose populations are usually predominately female. Most offspring have parthenogenetic replicas of the maternal genotype, but environmental deterioration triggers the formation of diapausing embryos (BANTA and BROWN 1929; BANTA 1939). In most species this requires sexual reproduction. Only the sexually produced ("ephippial") embryos can survive desiccation or freezing, so that each population must pass, from time to time, through sexual reproduction. The number of parthenogenetic generations between each sexual episode will depend on the stability of the environment and may vary from one or two to indefinitely many.

The opportunities for genetic change in an organism like *Daphnia magna*, which has periodic sexual reproduction, are very different from those in a purely apomictic organism. If we look at *Daphnia* as a sexual organism, then the entire

* Present address: Department of Applied Genetics, John Innes Institute, Norwich NR4 7UH, England.

time from the production of an ephippial egg to the time when the clonal descendants of that egg in turn produce ephippia, that is from meiosis to meiosis, is a single "lifetime." All the members of a clone from the first ex-ephippial female to her last surviving clonal descendant constitute the extended soma of a single genetic "individual." What are the characteristic features of *Daphnia* as compared with other sexual organisms?

(1) The average lifetime of a clone is much longer than that of a single animal. The temporal grain of the environment is correspondingly finer: a clone in a permanent habitat may persist through many annual cycles and see these as fine-grain changes.

(2) A clone is iteroparous. Matings may be "selfed" (with males of the same clone) or outcrossed. Random mating is unlikely, as genotypes commonly differ in the timing and extent of sexuality. Generations may in general be overlapping, even in intermittent populations because of the indefinite length of diapause.

(3) The effective population size for sexual reproduction is relatively small. It may be considerably less than the actual number of clones because of the variance in offspring number due to differences in clone abundance and sexual response, and possibly because of enhanced selfing if the times of male and of sexual egg production are genetically correlated.

(4) Selective coefficients per generation may be very large. Fecundity differentials may be very much greater than is practicable in a conventional sexual species. Fitness differentials will also be magnified; a slight disadvantage in each parthenogenetic generation will, if prolonged indefinitely, be effectively lethal. This strong selection can lead to extreme departures from single- and multiple-locus equilibrium during each generation. To the extent that the selection is typical in kind, although exaggerated in magnitude, we may expect its effects in *Daphnia* to be extreme manifestations of the effects of selection in any sexual population.

(5) The mutation rate may be high. There are on average many more mitoses or pseudo-mitoses between each meiosis than in most sexual organisms. If the mutation rate per parthenogenetic generation is similar to that in a normal sexual generation, then the overall rate of mutation per sexual cycle will be higher than usual. There is no evidence on parthenogenetic mutation rates in any species, so far as I know. A high mutation rate would increase the accumulation of recessive deleterious genes, increasing the mutational load at meiosis. Some of these mutations may be carried to appreciable frequency in the population by selection on associated loci. Deleterious mutations with some dominance will, however, be selected against as soon as they arise.

In conclusion, the idiosyncrasies of *Daphnia*'s life history lead to a distorted relationship between the parameters of selection, mutation and inbreeding, but the ensuing effects may be present in a more moderate, but still significant, form in species without parthenogenesis.

Electrophoresis provides convenient markers for the study of genetic structure. Such markers were first examined in *Daphnia* by HEBERT (1974a,b,c,d; HEBERT and WARD 1976; HEBERT, WARD and GIBSON 1972). His extensive studies cover

many populations and provide evidence of great heterogeneity among populations, persistent Hardy-Weinberg deviations, frequency changes indicating selection, and fecundity differences between genotypes. In most populations, however, changes were observed at a single locus, so that information on the simultaneous behavior of several loci and of their relationships is lacking. One aim of the present study was to provide this added dimension, which helps to distinguish between locus-specific effects and general genomic effects.

MATERIALS AND METHODS

In the accompanying paper (YOUNG 1979), I described the population of *Daphnia magna* in Pond IV at the Plant Breeding Institute, Trumpington, near Cambridge, England. The present paper concerns Pond III, a smaller pond to the north of Pond IV, and separated from it by a stand of sedge growing on slightly higher ground. The two ponds became interconnected when the water level rose sufficiently, which probably occurred for several weeks or months during the spring of most years. However, in order to study changes in the *D. magna* population of Pond III without contamination from Pond IV, a dam was constructed in January, 1974, across the highest point between the two ponds. This dam was made of clay stabilized with wooden stakes and interwoven branches, and provided an additional barrier about 30 cm high. This was sufficient to prevent any mixing during the spring of 1974, and it was not until January, 1975, that the water level rose sufficiently to permit water to flow from Pond IV to Pond III and thence out through a culvert at the north end. Pond III was six meters by three meters at its lowest level (September, 1974) and 36 meters by ten meters at its highest (February, 1975). The maximum depth fluctuated from 30 cm to 120 cm.

Population sampling techniques were similar to those described for Pond IV (YOUNG 1979) although it was not possible to estimate the population density directly because Pond III was very shallow and strewn with branches. Fecundities and genotype frequencies were estimated from samples of about 200 adult females, except that the first sample of 1974 comprised only 96 animals.

RESULTS

Population size and reproduction: In the summer of 1973, *Daphnia curvirostris* was very abundant and *D. magna* was abundant, with small numbers of *D. pulex*, *Ceriodaphnia quadrangula*, copepods and ostracods. During the autumn, dense sexual swarms occurred in part of the pond (YOUNG 1978), but the samples described in the present paper were collected from a region without swarms. At the beginning of December, 1973, the pond was frozen to a depth of about six centimeters for about a week, and after melting it contained no Cladocera and few other animals. A week later several tons of sugar beet sludge were dumped in the pond, extinguishing the remaining animal life. The first *Daphnia pulex* reappeared at the end of January, 1974, and the first *D. magna* in mid-February, although very few were caught until mid-April. Since extensive netting during December and January failed to produce a single daphnid, it is assumed that these species survived only in the form of diapausing embryos (*i.e.*, the sexually produced stage), produced during the preceding summer or some previous season, and stimulated to hatch by the spring conditions. The genetic data support this idea. *Daphnia curvirostris* was very scarce during 1974 and 1975.

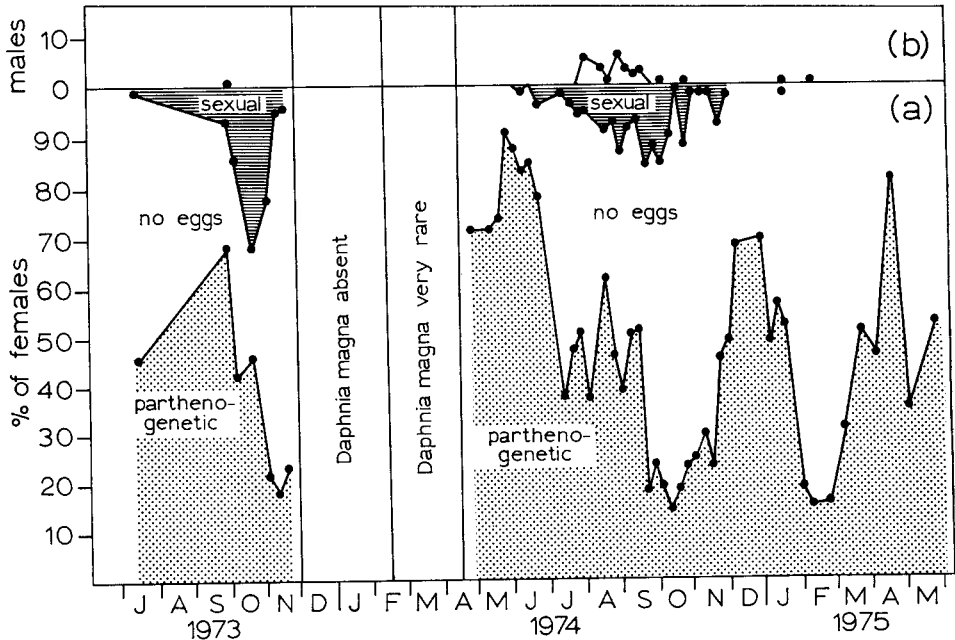


FIGURE 1.—Reproductive distribution in Pond III. In the lower graph (a), adult females are partitioned into three classes: the stippled area below the lower line represents females carrying parthenogenetic eggs, the shaded area above the upper line represents females with sexual eggs, and the area between these lines represents nonreproductive females. The upper part of the graph (b) records the number of adult males per 100 adult females.

During the spring and summer of 1974, *Daphnia magna* increased to a very high density. Numbers decreased rapidly during October and November, but began to increase again during December and throughout the winter. In the first half of 1975, the *D. magna* population was substantial although never approaching the levels of 1974.

The pattern of reproduction is shown in Figure 1. There was a burst of sexual egg production at the end of 1973 and a continuous low level of sexuality throughout 1974, although males were always rare.

Electrophoretic polymorphism: In view of the close proximity of the two populations, it is not surprising that the genetic variation in Pond III (the subject of this paper) showed a considerable similarity to that in Pond IV (YOUNG 1979). In particular, *Est*, *Mdh* and *Got* were all polymorphic for the same arrays of alleles described for Pond IV (YOUNG 1979), and the comments on other enzymes in that paper apply also to Pond III. However, although the same alleles were involved in both populations, their genotypic distribution was quite different. Pond III showed a more complex pattern of variation, and one which changed over time in a fairly regular way.

The genotype frequencies at each of the three polymorphic loci are shown in Figures 2 to 4; they contrast with the Pond IV data in two salient ways. In the

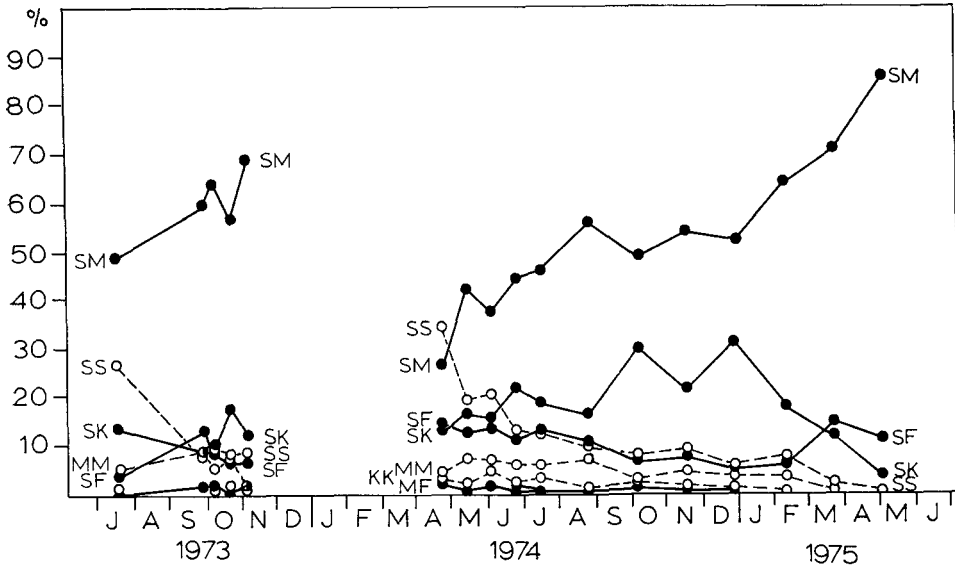


FIGURE 2.—*Est* genotype frequencies in Pond III (●, heterozygotes, ○, homozygotes).

first place, there was considerably more genetic variety; homozygotes in particular were much more in evidence. Second, there were sustained changes over time. The most interesting changes are those which took place during 1974; sampling in 1973 began in midseason and provides useful confirmation of the 1974 picture. Changes during 1975 were heavily influenced by migration from Pond IV.

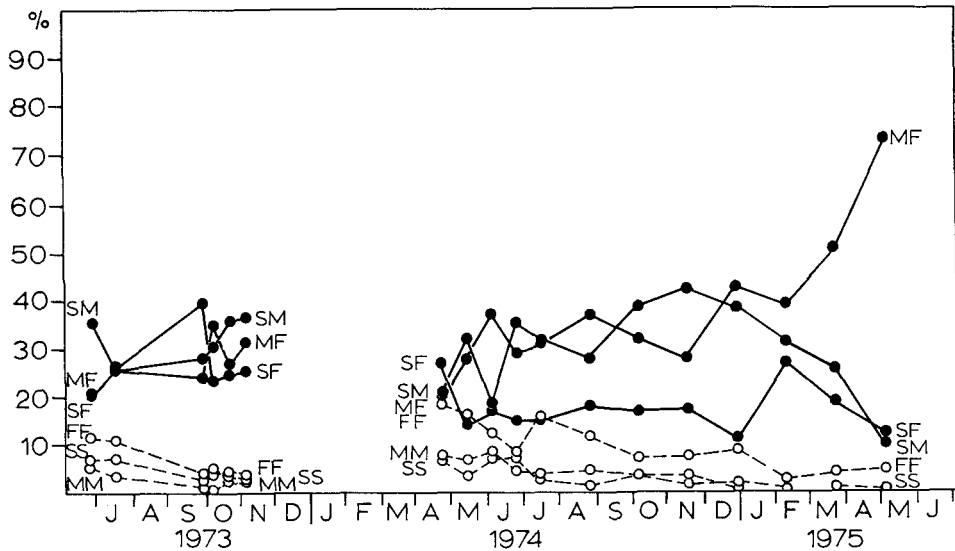


FIGURE 3.—*Mdh* genotype frequencies in Pond III (●, heterozygotes; ○, homozygotes).

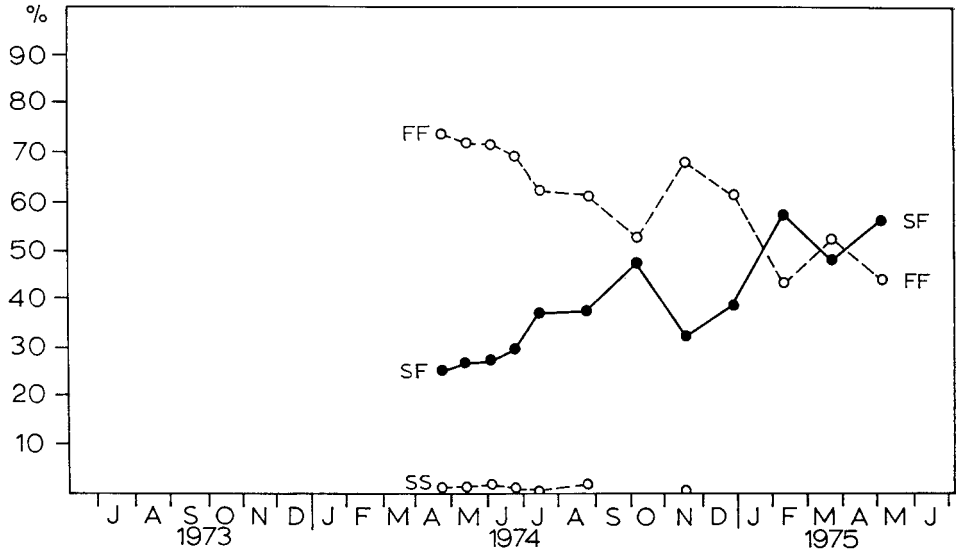


FIGURE 4.—*Got* genotype frequencies in Pond III (●, heterozygotes; ○, homozygotes).

Similar changes occurred at all three loci during 1974. Genotype frequencies in the first sample, soon after the population reappeared, were at or near Hardy-Weinberg equilibrium. During the course of the year a considerable deviation built up at each locus. In each case, this was largely a reflection of the very marked decline in homozygote frequencies (Figure 5). These trends are also

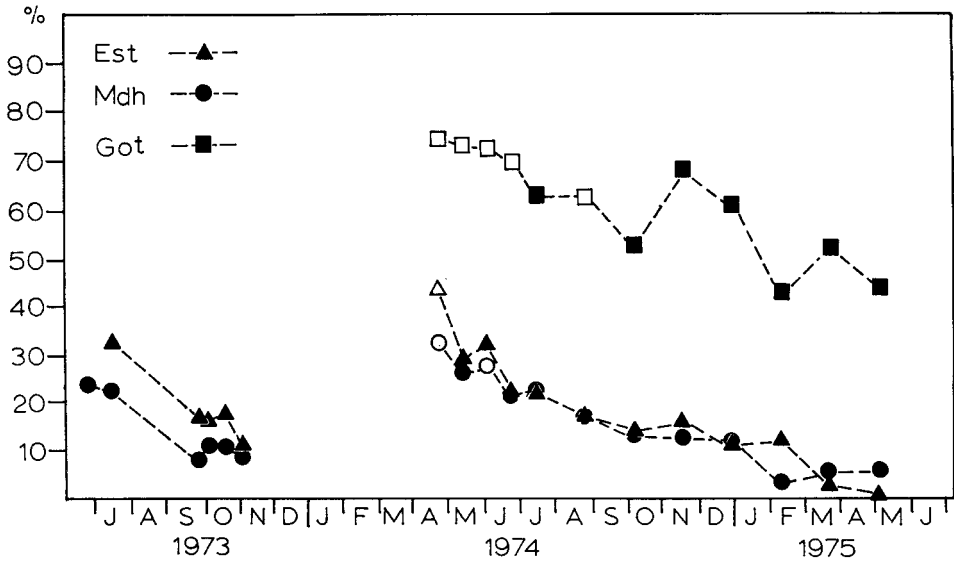


FIGURE 5.—Total homozygote frequencies at *Est* (▲, Δ), *Mdh* (●, ○), and *Got* (■, □) in Pond III. Open symbols represent samples that approximate to Hardy-Weinberg equilibrium, while solid symbols represent significant deviations ($p < 0.01$ by *G*-test).

apparent in 1973. The changes were primarily in genotype frequencies and not in gene frequencies, which changed much less markedly.

Although the overall pattern at all loci is of declining homozygote and increasing heterozygote frequencies, the heterozygotes at each locus did not all behave alike, and neither did homozygotes. In particular, both *Est*-SF and *Mdh*-SF declined in frequency.

Each individual was examined at all three of the polymorphic loci (Table 1) and nearly all combinations of the commoner genotypes were found. Altogether during the study, the examination of just three polymorphic loci allowed a total of 94 distinct genotypes to be identified. There was a small but consistent association between loci (Figure 6, in which $\ln A$ is a measure of association between the commonest genotypes at each locus, as defined by YOUNG 1979). The sudden increase in the *Est*-*Mdh* association in 1975 probably reflects migration from Pond IV.

Differences in fecundity between genotypes: If fecundity differences were the main cause of the observed changes in genotype frequency, then the genotype frequencies at a given time should reflect the frequencies in embryos being carried in brood pouches at some previous time. The latter frequencies can be calculated from the genotypes and brood sizes of the mothers. The frequencies of certain genotypes in adults and in embryos are presented in Figure 7.

Got-FF had below average fecundity in the earlier samples, and also declined in frequency. *Est* showed a surprisingly good correlation between fecundity and frequency change. *Mdh*-MF showed a poor or even negative relationship between

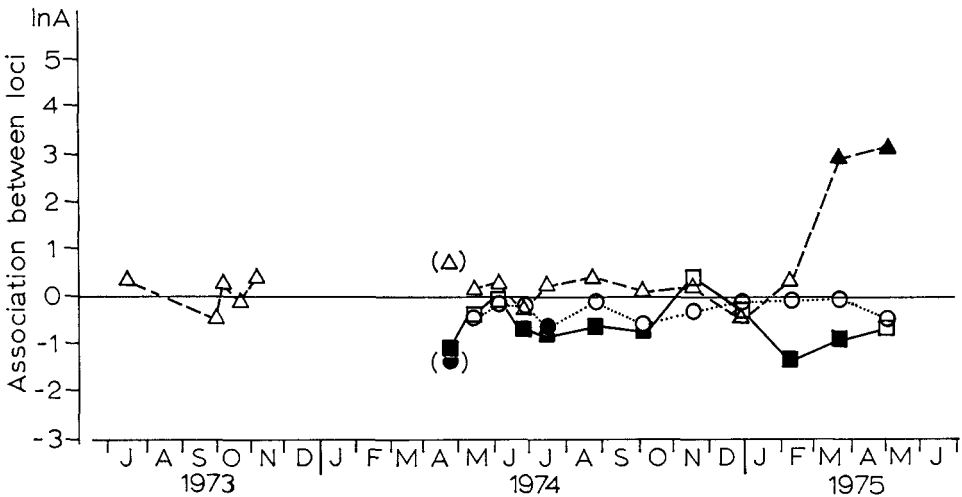


FIGURE 6.—Association between loci in Pond III. The log cross-product ratio, $\ln A$, defined as in YOUNG (1979), is a measure of the deviation from random association between genotypes at two loci; $\ln A$ is zero for random association and positive if the frequency of the commonest genotype at one locus is highest amongst individuals that have the commonest genotype at the second locus. The associations shown are: Δ \blacktriangle *Est*-SM and *Mdh*-MF; \circ \bullet *Mdh*-MF and *Got*-FF; \square \blacksquare *Got*-FF and *Est*-SM. Solid symbols differ significantly ($p < 0.05$ by *G*-test) from random association.

TABLE 1
Numbers of each three-locus genotype in samples from Pond III

Gen	EF												SF												Total												
	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM	SS	MM															
1973	6	4	4	8	25	6	2	1	5	4	3	12	34	17	29	3	8	7	7	1	3	3	No Gen data*												10		
Jul 16	1	2	4	4	4	2	4	3	4	1	1	3	36	50	24	7	4	7	1	3	13	6	No Gen data*												6		
Oct 1	3	4	4	6	4	1	4	2	3	2	51	32	49	2	6	3	8	2	2	6	7		No Gen data*												12		
Oct 8	1	4	3	7	3	5	1	1	1	2	50	35	32	1	15	8	13	3	2	3	7		No Gen data*												17		
Oct 22	1	2	3	11	1	1																	No Gen data*												10		
Nov 5	1	2	3	11	1	1																	No Gen data*												215		
1974	5	2	6	3	13	1	1	1	1	1	3	3	3	4	1	2	2	4	4	3	3		No Gen data*												93		
Apr 23	6	2	7	6	5	1	1	1	1	1	3	6	21	9	14	3	9	2	9	7	1	2	4	No Gen data*												13	
May 14	3	2	7	3	8	9	4	1	2	3	6	4	13	5	20	3	2	2	10	2	2	3	9	1	No Gen data*												192
Jun 3	4	3	5	7	1	1	1	1	1	4	2	23	8	13	2	14	5	13	4	5	1	2	1	No Gen data*												192	
Jun 24	2	1	4	6	4	2	1	1	2	3	4	7	12	7	13	3	13	3	9	5	6	4	2	1	No Gen data*												192
Jul 15	2	1	2	6	2	1	1	2	1	3	4	17	11	22	3	9	2	11	5	4	4		No Gen data*												191		
Aug 27	1	2	2	6	2	1	1	2	1	2	3	17	8	11	2	18	2	10	5	4	1		No Gen data*												13		
Oct 7	4	1	1	3	6	1	2	3	1	2	6	31	12	23	2	10	1	8	3	3	1		No Gen data*												187		
Nov 18	1	1	3	6	1	2	1	1	1	2	7	17	8	20	2	15	1	21	4	1			No Gen data*												8		
Dec 30	1	3	1																				No Gen data*												5		
1975	1	1	1	2	2	1	2	1	2	1	4	12	9	14	11	14	3	4	1	3	4	1	No Gen data*												192		
Feb 11	1	1	1	2	2	1																	No Gen data*												2		
Mar 24	1	1	1	2																			No Gen data*												189		
May 6	1																						No Gen data*												1		

* Individuals sampled during 1973 were typed at *Esr* and *Mdh* only.

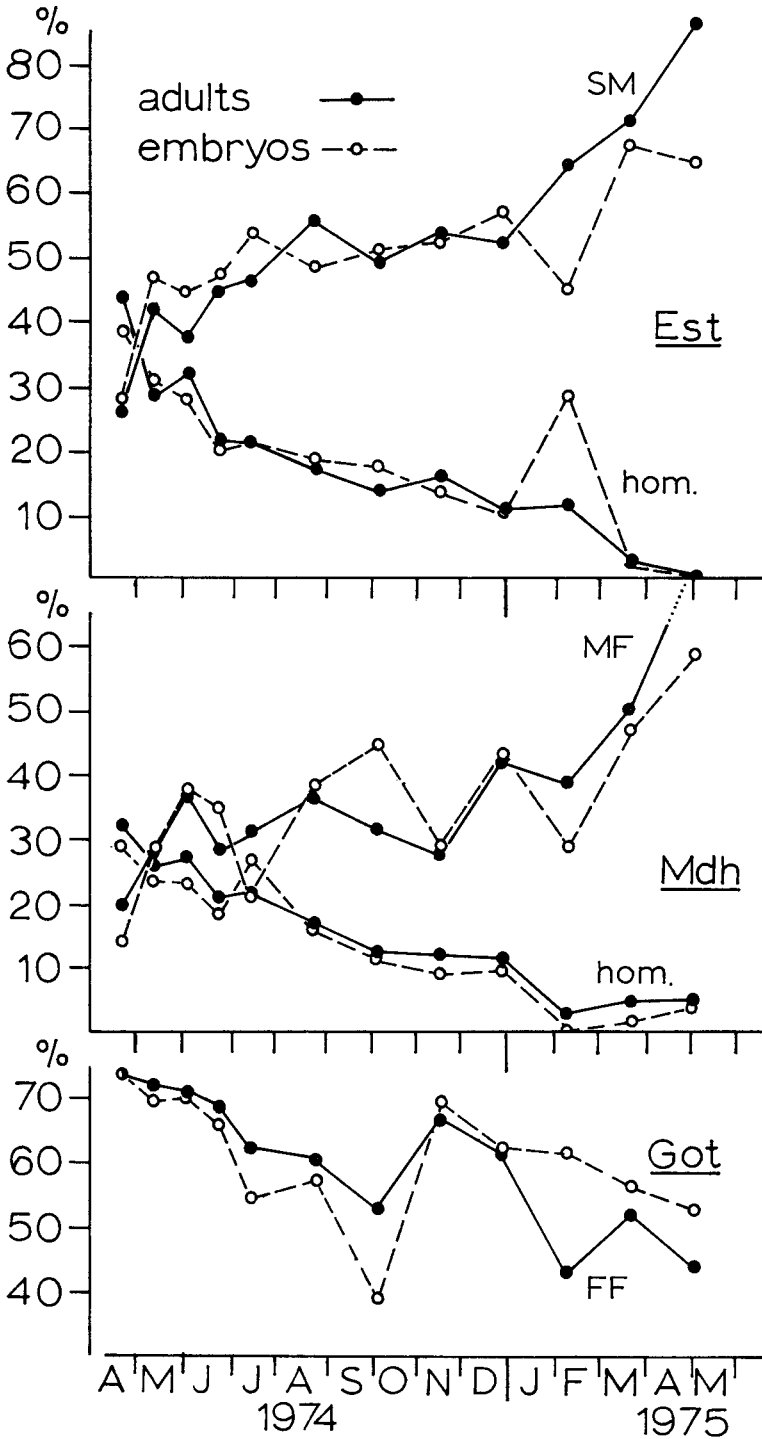


FIGURE 7.—Genotype frequencies amongst adult females (●) and amongst their embryos (○) in Pond III. Frequencies of the commonest genotypes at each locus are shown, together with the total homozygote frequencies for *Est* and *Mdh*.

fecundity and subsequent change, but *Mdh* homozygotes had below average fecundity in nearly all samples and were of course declining in frequency. Qualitatively, then, fecundity differences were often in the right direction to cause the frequency changes. It would be valuable to check whether they were also of an appropriate magnitude, but unfortunately the relationship between fecundity differences and frequency changes is heavily dependent upon parameters that were not measured and some that cannot readily be measured. These include population growth, and development and death rates, which may be genotype specific.

DISCUSSION

The cause of heterosis: There can be no doubt that the genotype frequencies at the observed enzyme loci were affected by selection. Not only did large frequency changes occur despite large population size (Figures 2 to 4), but genotypic classes differed in fecundity (Figure 7). The selection was "heterotic" in that increasing excesses of heterozygotes were observed at each of the three loci. The question is whether the fitness differentials were intrinsic effects of genotypes at the observed loci themselves or reflected more extensive genomic differences. In the latter case, any selection on an enzyme locus itself might be swamped by selection on one or more unseen loci in disequilibrium with it.

A single-locus explanation of the observations requires large selective coefficients, usually favoring heterozygotes, at each of the loci. To see whether this is reasonable, we may examine HEBERT'S (1974b,c) extensive data from other populations. He distinguished between intermittent populations, which are re-established annually from ephippia, and permanent ones, in which parthenogenesis proceeds uninterrupted from year to year. In general, HEBERT found Hardy-Weinberg equilibrium in intermittent populations, with small erratic frequency changes during the season. In permanent populations, large heterozygote excesses were common. These facts are consistent with weak heterosis at the observed loci, such that the effects of selection are barely noticeable during one season, but become significant after prolonged parthenogenesis. In contrast, dramatic changes were obvious in Pond III within a matter of weeks. Was the environment of Pond III so unusual that selection was greatly intensified at each of the loci examined? There was no indication that it was.

An easier explanation lies in the unusual genetic structure of the population. Although the Pond III population was intermittent during the study period, it had probably been extensively colonized in the past from Pond IV, an adjacent large pond with a large, permanent *Daphnia* population (YOUNG 1979). In addition, the Pond III population itself was not regularly intermittent: a temporary eclipse such as that of 1973–1974 probably occurred only in occasional years. The extant population may therefore have been the result of prolonged parthenogenesis, during which selection, mutation and drift caused considerable genetic divergence between lines and between chromosomes within each individual. The surviving clones would be highly selected for parthenogenetic success, but might incorporate heterosis, epistasis and recessive defects that would disfavor segregants and recombinants upon meiosis. It would not be surprising, therefore, if

after sexual reproduction the fittest offspring were those most similar to their parents. This seems to have been the case in Pond III: during 1974, genotype frequencies moved towards those prevailing in late 1973. If there were substantial disequilibrium among loci, then this effect may be observed at any locus whether or not the locus itself contributes materially to the selection. It is evident from Figure 6 that some disequilibrium exists between the observed loci, though far less than in the permanent Pond IV population (YOUNG 1979).

If selection were acting on one or many unseen genes, why should the effect on the observed loci be primarily heterotic? It appears that if sufficient loci are selected, then overall heterosis is the most likely consequence. The ways in which genotype frequencies at one locus may be influenced by selection at other loci have received increasing attention, particularly since electrophoresis has made it clear that genomes are densely studded with polymorphic loci. Linkage makes it difficult to isolate the fitness effect of a single locus even in sexual outbreeders (FRANKLIN and LEWONTIN 1970), and the problem must be far worse in *Daphnia*. These interlocus effects depend on gametic disequilibrium (but not necessarily on linkage), which may arise through epistasis or stochastic effects at low population sizes or low gene frequencies. Directional selection at one locus can change gene frequencies at linked loci and greatly magnify linkage disequilibrium, an effect called "hitchhiking" by MAYNARD SMITH and HAIGH (1974). Heterozygote superiority at a locus will cause similar heterosis to appear at loci in disequilibrium with it ("associative overdominance", FRYDENBERG 1963). OHTA and KIMURA (1970) pointed out that this effect is increased by strong selection and high variance in offspring number. These features are characteristic of *Daphnia* as a sexual organism. Selection against a recessive allele will usually cause heterozygotes at associated loci to appear intermediate, but if two or more such selected loci are in repulsion disequilibrium, the overall effect may be heterotic. This phenomenon has long been recognized as a possible source of hybrid vigor (JONES 1917) and might be termed "synthetic heterosis" since none of the loci are, individually, heterotic. SVED (1972) has discussed this "heterosis at the level of the chromosome." Because of this effect, disequilibrium between loci will lead to heterosis even if single gene effects are not heterotic, and conversely the observation of heterotic frequency changes is not, *per se*, evidence for heterotic loci [in contrast to the implication of BERGER's (1976) discussion of "the special case of *Daphnia*"]. I emphasize that I am not claiming that the enzyme loci in *Daphnia* are necessarily neutral, but that even if they were, we should expect to see heterosis. The associative effects of a single selected locus will be small under most circumstances, but when a number of loci are in disequilibrium, their joint effect may be much greater.

It is of course frustrating to "pass the buck" of selection to unknown loci. After all, any data can be fitted with the aid of sufficient unobservables. However, the effects I have discussed will occur whenever there is disequilibrium between loci, small effective population size (so that there is appreciable inbreeding), and a sufficient number of selected loci. These conditions were fulfilled in Pond III. Modest but significant association between loci was detected. The number of clones appeared to be surprisingly high, but inbreeding would be enhanced by

the variance in clone sizes and by genotypic differences in the time of sex (YOUNG, in preparation), as mentioned above. Finally, independent selective effects were observed at each of the three marked loci (associations between the loci did not change), so that there must be many selected genes in the genome.

Hence, "synthetic heterosis" and "associative overdominance" are bound to exist in this population and seem to be the most plausible explanation for the extraordinarily rapid selection observed. There are a number of reasons, discussed in the Introduction, why their effect should be greater in *Daphnia* than in many other organisms, but *Daphnia*'s population genetic parameters differ only in magnitude, and not in kind, from those of other sexual organisms. Whenever apparent heterosis is observed, it is always necessary to consider the possibility of genomic disequilibrium.

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Corresponding editor: R. W. ALLARD