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SHORT PAPERS

Natural selection for enzyme variants among parthenogenetic *Daphnia magna*

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SUMMARY

The frequencies of genetically determined electrophoretic variants of two enzyme systems in the parthenogenetic crustacean *D. magna* have been followed in two isolated populations. In both populations a marked excess of heterozygotes was found in the later samples. It is concluded that the observed changes in gene and genotypic frequencies are due to natural selection as both migration and genetic drift can be excluded.

INTRODUCTION

We have investigated polymorphisms for a number of enzyme variants in the crustacean *Daphnia magna* Straus. The results demonstrate for the first time enzyme polymorphisms in an organism with parthenogenetic multiplication. They also provide evidence for selective changes in the frequencies of genetically determined enzyme variants in populations whose sizes and relative isolation in freshwater ponds exclude migration or genetic drift as causes for the observed changes in gene and genotypic frequencies (see Lewontin, 1967).

1. MATERIALS AND METHODS

In most conditions *D. magna* is viviparious, with a generation time of approximately 2 weeks. At such times reproduction is by ameiotic parthenogenesis (Mortimer, 1936; Ojima, 1958). These cytological observations have now been confirmed genetically as heterozygotes show no segregation with respect to the alleles described here. In adverse conditions male offspring are produced and sexual reproduction is possible, each female developing two sexual eggs.

We have studied populations in two permanent ponds near Cambridge (at Audley End and Harlton) which have, with the exception discussed below, been reproducing parthenogenetically. These two populations are being regularly sampled and assayed for electrophoretic variants of six enzymes. In the present paper we will only discuss the results for esterase variants in the Audley End population and for soluble malic dehydrogenase variants in the Harlton population.

Crude extracts of single adult females were assayed on 7 % polyacrylamide gels. The gels were stained for malic dehydrogenase in a solution containing, per ml of distilled water, 0·5 mg NAD, 0·25 mg Nitro Blue, 0·1 mg phenazine methosulphate and 2 mg sodium malate. For esterases the gels were incubated in Tris malate buffer, pH 5·3, and

Table 1. *Malic dehydrogenase variants at Harlton*

		Genotypic frequencies			Gene frequency	Probability of fit to Hardy–Weinberg proportions
	n	FF	FM	MM	F	
May	188	0·23	0·47	0·30	0·47	$P > 0{\cdot}5$
June	263	0·37	0·47	0·16	0·61	$P > 0{\cdot}9$
July	310	0·48	0·43	0·09	0·69	$P > 0{\cdot}9$
August	460	0·36	0·55	0·09	0·64	$P < 0{\cdot}001$
September	294	0·26	0·69	0·05	0·61	$P < 0{\cdot}001$
October	368	0·28	0·67	0·05	0·62	$P < 0{\cdot}001$
November	284	0·25	0·71	0·04	0·61	$P < 0{\cdot}001$

stained in a solution containing, per ml of the buffer, 0·5 mg α naphthyl acetate and 0·1 mg Fast Red T.R. Salt.

The electrophoretic patterns of esterase and soluble malic dehydrogenase were the same in sexual and parthenogenetic phases and did not vary in different tissues of the same individual. The electrophoretic variants described have been shown by sexual crosses to be produced by alleles. These experiments will be described in detail elsewhere.

2. RESULTS

The *D. magna* population at Harlton was polymorphic for fast and medium electrophoretic variants of malic dehydrogenase. A slow electrophoretic variant of this enzyme occurs in another population. The Harlton population has been sampled twice each month since May 1970.

The results (Table 1) show that, at the time of the first samples in May, the frequencies of the three genotypes were in Hardy–Weinberg proportions. The maintenance of these proportions until August, despite a significant increase in the frequency of the F allele, can only be explained by assuming that the heterozygote had a fitness rather precisely intermediate on a multiplicative scale between those of the two homozygotes. The genotypic frequencies in the August and later samples were in marked contrast, and were significantly different from Hardy–Weinberg proportions. There was a marked excess of heterozygotes. It is clear that the MM genotype was at a selective disadvantage throughout the sampling period.

It is known that the number of broods and the number of parthenogenetic young produced in each brood vary with environmental conditions (Berg, 1931). For example, starved females produce few or no offspring, whereas well-fed individuals produce many. The females in the August and later samples were classified according to the number of embryos they carried. Using these data the relative fecundities of each genotype were estimated (Table 2). The fecundity of the heterozygotes in the August and September samples was greater than the fecundities of the homozygotes. At this time there was an increase in the proportion of heterozygotes in the population. During October and November the fecundities of the FF and FM genotypes were very similar and the proportion of heterozygotes did not increase further. Such a correlation between fecundity and genotypic frequencies would be expected if fecundity were the main determinant of fitness. Females do produce more than one brood, and the relationship between our fecundity data and fitness estimates would be obscured if females of different genotypes differed either in the interval between brood depositions or in developmental rate of embryos. However, no such differences are suggested by our data, for during periods of stable gene frequencies no significant differences in relative fecundity have been observed (see Tables 1

Table 2. *Relative fecundities of malic dehydrogenase variants at Harlton* 1970

	Relative fecundities		
	FF	FM	MM
August	0·36	1·00	0·50
September	0·48	1·00	0·23
October	0·95	1·00	0·79
November	0·96	1·00	0·48

Table 3. *Esterase variants at Audley End*

	n	SS	FF	MM	SF	SM	FM	S	F	M
20 July	120	—	0·06	0·26	0·20	0·01	0·47	0·10	0·40	0·50
6 Aug.	194	—	0·03	0·09	0·12	0·01	0·75	0·07	0·46	0·47
13 Aug.	144	—	0·04	0·14	0·04	—	0·78	0·02	0·45	0·53
26 Aug	175	—	0·04	0·06	0·03	0·01	0·86	0·02	0·49	0·49
4 Sept.	156	—	0·02	0·09	0·01	0·01	0·87	0·01	0·46	0·53
August										
* Females with:										
0 embryos	163	—	0·04	0·13	0·07	0·01	0·74	0·04	0·45	0·51
1 or 2 embryos	143	—	0·05	0·06	0·03	0·01	0·85	0·02	0·49	0·49
3 or 4 embryos	100	—	0·01	0·05	—	—	0·94	—	0·48	0·52
13 Aug.										
Females with sexual eggs	33	—	0·09	0·58	0·03	—	0·30	0·01	0·26	0·73

* The first 74 females assayed from the 6 August sample were not classified for embryo number.

and 2). The short generation time of *Daphnia magna* means that differential fecundities would be rapidly reflected in genotypic frequencies. Many ecologists have suggested that fecundity is a fitness component of major importance in opportunistic species such as *Daphnia* (e.g. Margalef, 1963).

The Audley End population was polymorphic for three esterase alleles which were classified electrophoretically as fast, medium and slow. The six samples of this population obtained over a 7-week-period (Table 3) show that the frequency of the S allele decreased. The FM heterozygote increased in frequency until there was a significant excess, and the genotypes MM and SF decreased in frequency. In August there was a significantly higher proportion of FM heterozygotes amongst females carrying three or more embryos than amongst females with one or two embryos ($\chi_1^2 = 5{\cdot}08$; $0{\cdot}05 > P > 0{\cdot}01$) which in turn had a higher proportion of FM heterozygotes than females without embryos ($\chi_1^2 = 7{\cdot}96$; $0{\cdot}05 > P > 0{\cdot}01$).

In the second August sample a small proportion of females containing sexual eggs was found and these were scored separately. The genotypic frequencies were in marked contrast to those in parthenogenetic females (Table 3), and it is interesting that the MM genotype, whose frequency had been reduced by selection, was at high frequency in sexual females.

Since both ponds have maintained breeding populations of many thousands of individuals, genetic drift can be excluded as the cause of the observed changes in gene and genotypic frequencies. Furthermore, extensive sampling of the Harlton pond revealed no spatial heterogeneity in gene or genotypic frequencies. The Audley End pond is considerably larger in area, but again there was no spatial heterogeneity in gene frequencies.

There was, however, some heterogeneity in genotype frequencies, for the frequency of FM heterozygotes, although in excess of Hardy–Weinberg proportions in all samples, was significantly less in two additional samples taken in August from different parts of the pond to the usual sampling site.

We conclude that the observed changes in both ponds were due to natural selection. The magnitude of these changes over the sampling period might be taken to indicate that the selective differentials between the enzyme variants were large. However, with given differences in, for example, fecundity, and providing that the required genotypes are present in the population, parthenogenetic multiplication makes possible more rapid changes in genotype frequency than sexual reproduction, as the favoured genotypes under sexual reproduction will generate progeny of other genotypes.

These considerations further suggest that populations multiplying parthenogenetically may be more prone to lose alleles through gene fixation than are sexually reproducing populations. This means, as White (1970) has suggested, that populations which maintain gene frequencies by heterosis during parthenogenetic multiplication might have an evolutionary advantage. The strong selection for heterozygotes that we have found in both populations is in agreement with this view.

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