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DYNAMICS OF AN ENZYME POLYMORPHISM IN THE ISOPOD, *SPHAEROMA RUGICAUDA* (LEACH). I. TEMPORAL VARIATION IN GENOTYPE FREQUENCIES

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SUMMARY

A 27 month study (November, 1978 through January, 1981) of the diallelic enzyme locus, phosphoglucose isomerase (Pgi), in the estuarine isopod *Sphaeroma rugicauda* (Leach) revealed seasonal changes in the frequencies of the three genotypes (fast/fast, fast/slow and slow/slow). A comparison of genotype frequencies in the newly-released offspring (August) to the frequencies in the same animals ten months later during the breeding season (June), revealed a decline in heterozygote frequency and a corresponding increase in the frequency of the s/s homozygote both of which appeared to be initiated at the onset of winter, a period characterised by low temperatures and salinity. Survivorship experiments in the laboratory suggested that the increase in frequency of the s/s homozygote, at least, could be explained by this genotype being favoured at low temperatures.

1. INTRODUCTION

Much of the work carried out on polymorphic enzyme loci has concentrated on studies of spatial variation. The primary aim of such studies being to correlate any observed spatial difference in gene or genotype frequency with environmental parameters in an attempt to discriminate between the selectionist and the neutralist position. However, the use of such correlates to provide evidence for the operation of natural selection suffers from two major disadvantages. Firstly, there is a tendency amongst workers to report only those studies where positive results were obtained, negative studies in which no clear pattern emerged rarely being reported. It is, therefore, difficult to ascertain how representative are these studies which show positive correlations between genetic variation and the environment. Secondly, the assumption is made that a non-random pattern of allelic variation implies that the organisms are adapted to different localities and further, that this pattern of variation represents a state of equilibrium. This assumption ignores the historical component of any pattern of distribution found in natural populations. For example gene frequency differences between populations could be explained by the foundation of each population by a small number of individuals (founder effect) and consequent genetic drift (Lewontin, 1974).

A far better method of investigating the operation of selection is to monitor seasonal changes in gene and genotype frequencies within a given natural population. Cyclic environmental changes are obviously a basic

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ecological factor in the life histories of many organisms particularly where these changes are abrupt or severe. A repeated cycle of genetic changes in a population closely following seasonal environmental changes would provide strong evidence for the operation of selective agents. Such studies on temporal genetic variation also have the added advantage that the genetic history of the population, which can be the cause of spatial variation, is irrelevant to the observed frequency changes.

Of those studies investigating temporal variation at enzyme loci, most show only intermittent or partial correlations with seasonal environmental changes. Berger (1971), for example, found that the frequency of the slow allele at the α-glycerophosphate dehydrogenase locus doubled over a 20-week period between mid-June and mid-November in four populations of *Drosophila melanogaster*. As this pattern was repeated over two years at two distinct localities, the operation of selective agents was considered the most likely explanation. A similar correlation between gene frequencies and environmental parameters was demonstrated at three loci (two esterases and a malate dehydrogenase) in the ant *Pogonomyrmex barbatus* by Johnson *et al.* (1969). Principle component analysis was used to compare the patterns of variability of the allelic frequencies with environmental factors (plant leaf areas, elevation, rainfall, temperatures and growing season). Significant correlation was particularly evident with respect to weather and the pattern of variability in both esterases, from which it was inferred that natural selection was important in determining the allele frequency patterns.

Such studies attract three criticisms. Firstly since we are concerned with genetic variation it is important to show that allozyme variation has a genetic basis; this is often not done. Secondly a correlation may imply selection but this is often not verified by the appropriate experiments. Thirdly, while the neutralist position may be refuted by the demonstration of some directional selection this is insufficient to explain how the variation is maintained in the population, as this requires special sorts of selection. A full defence of the selectionist hypothesis involves the measurement of net fitnesses over a complete generation and thus an investigation of the effects of selection at all stages of the life cycle (Lewontin, 1974). Practically this is not easy to achieve although the attempts by Christiansen *et al.* (1977) and Sassaman (1978) have met with some success.

The work described in these two papers is an analysis of components of fitness in an estuarine isopod. *S. rugicauda* is well suited to this type of investigation for the following reasons. It is abundant and easily sampled and has separate annual generations. Mating pairs can be easily sampled from the natural population and fecundities are easily estimated, as the animal is viviparous. It is also easily maintained and bred in the laboratory.

2. Methods

The saltmarsh from which samples of animals were taken was located at the eastern extremity of Alresford Creek in Essex (Map Reference TM 168 082194).

Random samples were taken every month from approximately the same region of the saltmarsh (see Edwards, 1981 for details). The samples were taken as the incoming tide rose on to the saltmarsh, as the animals were

found to be swimming actively during this period (West, 1964). The animals were then transferred to the laboratory and stored at −40°C until required for electrophoresis. Water temperature and salinity were also recorded on each sampling data. These two variables were chosen for study as they were easy to reproduce in laboratory experiments, as well as having been shown to be of significance in previous studies on *Sphaeroma* biology (Bishop, 1969; Heath, 1974; Khazaeli and Heath, 1979).

To ensure that the observed variation at the Pgi locus was genetically determined, pairs of animals were allowed to breed in the laboratory. Once offspring had been released, the parents and ten randomly chosen offspring were electrophoresed. The electrophoretic technique adopted for this study was horizontal starch gel electrophoresis, details of which are given in Edwards (1981).

For reasons which will become apparent on presentation of the frequency and environmental data, the survival of the three Pgi genotypes were compared at 3°C at three different salinities, 3‰, 12‰, and 35‰. Three experiments were set up using newly released offspring derived from an August sample, adults derived from a pre-winter November sample and a post-winter April sample. The animals used for these experiments were a sub-sample of the respective monthly sample and were, therefore, assumed to be composed of a similar proportion of each Pgi genotype as recorded for that month's main sample. Two hundred animals were used for each temperature/salinity combination the survivors being electrophoresed once approximately 50 per cent mortality had occurred.

3. Results

(i) *Crossing experiments*

Phosphoglucose isomerase (Pgi) is a dimer, the molecule being constructed of two sub-units. There are three electrophoretic phenotypes: a fast-migrating band (designated f/f); a complex of three evenly spaced bands, the centre one of which being the most intensively stained (f/s); and a slow-migrating single-band (s/s).

If the observed variation is the result of two alleles segregating at a Pgi structural locus, certain expectations must hold for the relationship between parental genotype and progeny genotype. For example, no progeny homozygous for one allele would be produced by a female homozygous for the alternative allele. Table 1 shows the data obtained for crosses between like and unlike homozygotes. The data for crosses between

TABLE 1

Data from crosses between Pgi homozygotes

Type of mating	No. of matings	No. of progeny	Progeny genotypes f/f	f/s	s/s
f/f×f/f	6	60	60	—	—
f/f×s/s	4	40	—	40	—
s/s×f/f	3	30	—	30	—
s/s×s/s	4	40	—	—	40

heterozygotes and homozygotes have been omitted as the results violate the expected ratios of Mendelian transmission of alternative alleles and will be discussed in the following paper. From the table it can be seen that the observed variability has a genetic basis.

(ii) *Frequency data*

The frequency data for the three *Sphaeroma* generations covered by this study are presented in table 2.

The monthly frequencies of each Pgi genotype over the 27 month study period were tested for homogeneity (27×2 contingency table). The results, shown in table 3, indicate that the frequency of the f/f homozygotes remain constant over this period, whereas the frequencies of the f/s heterozygote and the s/s homozygote show significant frequency changes. If the frequencies of the latter two genotypes are presented graphically (fig. 1) it is apparent that there is a marked seasonal pattern of variation. In particular the heterozygote frequency decreases gradually from a maximum in August of each year (when all offspring have been released) to a minimum in the following June breeding season. Conversely the frequency of the s/s homozygotes reaches a maximum at the onset of each breeding season, the increase appearing to be initiated in early winter (Nov–Dec).

TABLE 2

Pgi allele and genotype frequencies

Date	Sample size (N)	Allele frequencies f	s	Genotype frequencies f/f	f/s	s/s
11·78	260	0·6154	0·3846	0·3615	0·5077	0·1308
12	280	0·5696	0·4304	0·3500	0·4393	0·2107
1·79	261	0·5651	0·4349	0·3487	0·4329	0·2184
2	265	0·5830	0·4170	0·3585	0·4490	0·1925
3	284	0·5599	0·4401	0·3099	0·5000	0·1910
4	280	0·5196	0·4804	0·2607	0·5179	0·2214
5	294	0·5799	0·4201	0·3912	0·4081	0·2007
6	278	0·5953	0·4047	0·4101	0·3705	0·2194
7	289	0·5848	0·4152	0·3599	0·4498	0·1903
8	275	0·6091	0·3909	0·3600	0·5054	0·1346
9	278	0·5989	0·4071	0·3417	0·5144	0·1439
10	270	0·5352	0·4648	0·3000	0·5444	0·1556
11	257	0·6070	0·3930	0·3580	0·4980	0·1440
12	290	0·5655	0·4345	0·3517	0·4276	0·2207
1·80	267	0·5843	0·4157	0·3596	0·4494	0·1910
2	280	0·5893	0·4107	0·3786	0·4214	0·2000
3	298	0·5587	0·4413	0·3389	0·4396	0·2215
4	273	0·5696	0·4304	0·3590	0·4212	0·2198
5	250	0·6000	0·4000	0·4000	0·4000	0·2000
6	273	0·6007	0·3993	0·4213	0·3589	0·2198
7	264	0·5852	0·4148	0·3409	0·4886	0·1705
8	290	0·6104	0·3896	0·3517	0·5173	0·1310
9	277	0·6109	0·3899	0·3610	0·4982	0·1408
10·80	296	0·6081	0·3885	0·3682	0·4798	0·1520
11	200	0·6350	0·3650	0·3700	0·5300	0·1000
12	170	0·6177	0·3823	0·3882	0·4589	0·1520
1·81	248	0·5907	0·4093	0·3710	0·4395	0·1895

TABLE 3

Test for heterogeneity of genotype frequencies (χ^2 27×2 contingency tables)

Genotype	χ^2	df	p
f/f	32·82	26	n.s.
f/s	67·23	26	<0·001
s/s	58·69	26	<0·001

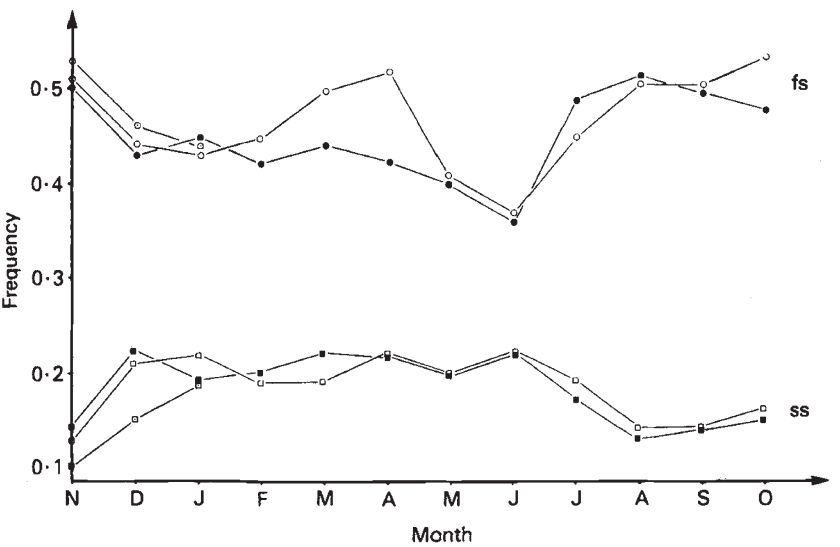

FIG. 1. Pgi-f/s and Pgi-s/s genotype frequency changes between Nov. 1978 and Jan. 1981. ○□ 1978–79, ●■ 1979–80, ⊙⊡ 1980–81.

(iii) *Environmental variables*

Over the period November, 1978 to January, 1981 (fig. 2) water temperature declined from June–July maxima of 20–27°C to minimum values of 2–7°C in the winter period (Nov-Feb), with a sharp decline in temperature of approximately 8–10°C between October–December.

Salinities showed a similar decline (fig. 3) from mid-summer (Aug–Sept) maxima of 14–16·5‰ to winter minima of between 1·7–5‰. The decline was, however, more gradual than the decrease in monthly water temperatures and did not exhibit an abrupt fall in October–December.

(iv) *Survivorship experiments*

The results for the experiments using animals derived from the post-winter April sample are shown in table 4. At all three salinities the genotype frequencies of the surviving animals did not significantly differ from the frequencies of the sample from which they were derived; nor did the combined data, suggesting that differential mortality had not occurred.

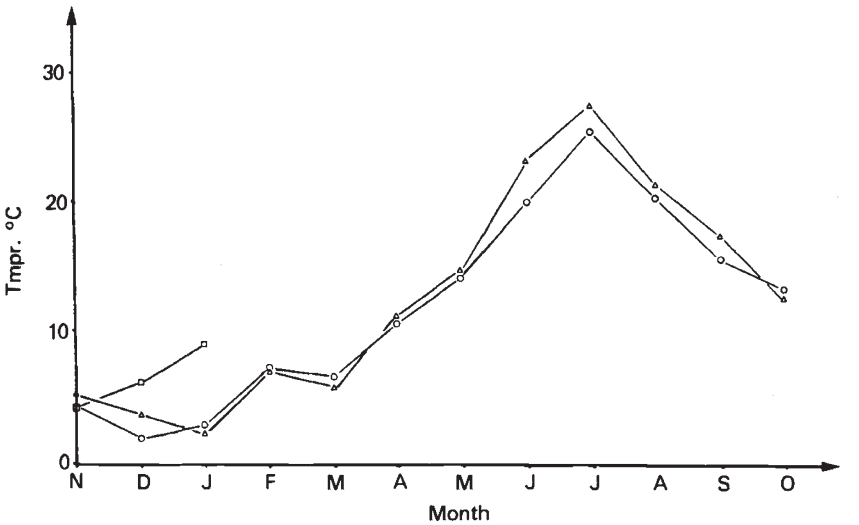

FIG. 2. Seasonal temperature variation at Alresford Creek (O–O 1978–79; Δ–Δ 1979–80; □–□ 1980–81).

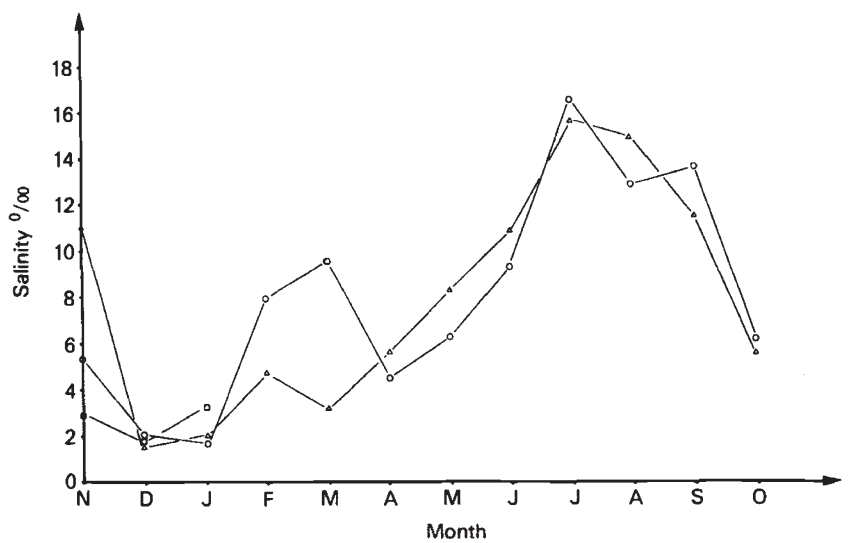

FIG. 3. Seasonal salinity variation at Alresford Creek (O–O 1978–79; Δ–Δ 1979–80; □–□ 1980–81).

However, the data from the experiments using newly born animals removed from the population in August showed that a significant change in genotype frequencies had occurred in the animals kept in water of 12‰ salinity. Although the changes in genotype frequency were not significant in the 3‰ and 35‰ experiments, in all three cases the frequencies of the s/s homozygote had risen. If the data are combined, the difference between the August sample frequencies and the experimental frequencies is statistically significant.

TABLE 4a

Genotype frequency changes in animals derived from pre- and post-winter samples when subjected to various salinities and low temperatures

April, 1980 sample (Genotype frequencies in main sample: f/f 0·3590, f/s 0·4212, s/s 0·2198)

Salinity	Survivor genotype frequencies f/f	f/s	s/s	χ^2 comparison between monthly sample and survivor freqs (2df)
3‰	0·3232	0·4344	0·2424	0·6240
12‰	0·3846	0·4519	0·1635	1·9250
35‰	0·3465	0·4456	0·2079	0·2506
Combined survivor genotype frequencies compared to April, 1980 frequencies (2df)				0·7672

TABLE 4b

Genotype frequency changes in animals derived from pre- and post-winter samples when subjected to various salinities and low temperatures

August, 1980 sample (Genotype frequencies in main sample: f/f 0·3517, f/s 0·5173, s/s 0·1310)

Salinity	Survivor genotype frequencies f/f	f/s	s/s	χ^2 comparison between monthly sample and survivor freqs (2df)
3‰	0·3093	0·4949	0·1958	3·7074
3‰	0·3143	0·4857	0·2000	4·4365
12‰	0·3056	0·4814	0·2130	6·4602*
35‰	0·3274	0·5045	0·1681	1·4154
Combined survivor genotype frequencies compared to April, 1980 frequencies (2df)				14·97***

TABLE 4c

Genotype frequency changes in animals derived from pre- and post-winter samples when subjected to various salinities and low temperatures

November, 1980 sample (Genotype frequencies in main sample: f/f 0·3700, f/s 0·5300, s/s 0·1000)

Salinity	Survivor genotype frequencies f/f	f/s	s/s	χ^2 comparison between monthly sample and survivor freqs (2df)
3‰	0·3438	0·4999	0·1563	3·3793
3‰	0·3684	0·4737	0·1579	3·7533
3‰	0·3846	0·5385	0·0796	0·7065
Combined survivor genotype frequencies compared to April, 1980 frequencies (2df)				2·512

As similar increases in the s/s homozygote were obtained at all three salinities, it was decided to repeat the experiment, keeping all animals at 3‰ in the cold room (the experimental conditions which nearest approximate those experienced by the animals during the winter at Alresford Creek). The animals used for this experiment were taken from the November monthly sample. In two out of three cases there were rises in the frequency of the s/s homozygote which were statistically non-significant, but in the third replicate the frequency of the s/s homozygote declined. Thus, the overall change in frequency from the combined data is non-significant.

4. Discussion

The results from the field data show that the frequencies of the three Pgi genotypes follow a seasonal pattern of variation. The size of the frequency changes and their regularity make it extremely unlikely that these are due to random events.

Providing, therefore, that there were no systematic errors in scoring the genotypes (a possibility discussed and discounted in Edwards, 1981) there are two possible explanations for the observed pattern of genotype frequency changes: (1) differential migration of genotypes, and (2) natural selection.

If there is spatial heterogeneity of morph frequencies or if different genotypes have different migratory tendencies, then migration could be a cause of frequency changes. Neither of these factors was investigated but several lines of evidence suggest that migration is a less likely explanation than selection. The area from which samples were taken had by far the highest density of isopods in the creek and was isolated to some extent from other suitable areas of marsh by unsuitable vegetation in which isopods were rare. Migration from sparsely populated areas into a densely populated one would have to be on a massive scale to change morph frequencies. Although the animals do swim, activity depends upon temperature and is low under winter conditions (Khazaeli, 1980). Khazaeli and Heath (1979) ruled out migration as a cause of colour morph frequency changes in the same population, since caged populations showed similar changes to the natural population. Finally the experimental results on mortality provide evidence for the alternative explanation. Since this study, further unpublished work has been done on mortality under winter conditions which agrees with the results presented here.

The survivorship experiments were set up to provide evidence in support of the alternative hypothesis that the observed genetic changes observed in the *Sphaeroma* population were due to seasonal differential mortality of Pgi genotypes. Studies by Harvey *et al.* (1973), Heath (1975) and Khazaeli (1980) have shown that the survival rate of *Sphaeroma* is reduced at the low temperatures normally experienced by the animals during the winter months in the wild. The results from the survivorship experiments indicate that animals derived from pre-winter samples contain individuals less able to cope with low water temperatures. In particular it would seem that the s/s homozygotes are at a selective advantage under these conditions. This hypothesis would fit well the observed frequency increase in s/s homozygotes in November–December when low water temperatures

are first recorded at Alresford Creek. The results from the experiments, however, fail to implicate salinity as a selective force as increases in s/s frequency are found at all three experimental salinities (3, 12 and 35‰). The genotype frequencies found in the swimming animals also do not show the decrease in heterozygote frequency observed in the wild. This is perhaps not surprising as the decline in heterozygotes continues during the spring and early summer and is, therefore, less closely associated with the winter period than is the increase in s/s homozygote frequency. Obviously other factors, as yet unknown, are involved in the decline in f/s frequency.

The data presented in this paper have provided evidence for the operation of seasonal zygotic selection on the *Sphaeroma* population. Before discussing the significance of this to *Sphaeroma* ecology and the mechanisms by which the polymorphism is maintained it is necessary to investigate the components of selection which may be in operation during the summer breeding season. This will be dealt with in the following paper.

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