GENETIC STABILITY OF POPULATIONS OF *PHYCIODES THAROS* (NYMPHALIDAE: MELITAEINAE)

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The elucidation of patterns of genetic variation between geographically separate populations is central to the study of evolution. Knowledge of such patterns contributes to an understanding of population structure and of the action of natural selection. Diverse selection pressures acting at different localities within a species' range may lead to local adaptation through genetic differentiation, and, in some instances, to speciation.

Electrophoretic separation of allelic forms of enzymes, or allozymes, has proven to be a useful tool for investigating genetic variability (Harris, 1966; Lewontin & Hubby, 1966; Selander & Johnson, 1973; Selander & Kaufman, 1973). Allozymes represent discrete phenotypic variation that can be statistically compared to a particular genetic model and can thereby be used for estimating gene frequencies at a number of different loci in natural populations. The frequencies thus estimated can be compared between populations, giving an indication of genetic differentiation at a number of enzyme-synthesizing loci. It is the purpose of the present study to assess genetic differences between populations of the butterfly *Phyciodes tharos* (Drury) by means of starch-gel electrophoresis of isozymes. The determination of such patterns not only provides information concerning the amount of population differentiation in this widely distributed species, but also may aid in understanding the effects of natural selection on protein-synthesizing loci in various populations.

While some authors contend that allozyme variability is selectively neutral (Kimura, 1968; King & Jukes, 1969; Kimura & Ohta, 1971), the existence of patterns such as clines along environmental gradients or genetic constancy over a wide geographic range seems to provide strong evidence for the adaptive nature of this variation.

P. tharos is distributed widely over most of North America. Although three subspecies have been named (dos Passos, 1969), the nominate *P. tharos tharos* (Drury) occupies all of the species' range in the United States east of the Rocky Mountains and shows very little geographic variation in phenotypic appearance (Klots, 1951). Despite this uniformity, the species occurs in a number of different habitat types. This, together with its wide geographic range, allows sampling from populations which can reasonably be assumed to be subject to very different

environmental selection pressures and in which any genetic differentiation likely to arise from such differences may be accentuated.

METHODS

Samples of *P. tharos* were obtained primarily from three field collections. One sample of 105 male butterflies was taken in open pine woods 6 km N of Silsbee, Hardin Co., Texas, on March 22, 1973. Two other collections were made in the vicinity of Ithaca, New York, on September 13, 1973. One of these (Brooktondale, N.Y.) included 55 individuals (38 males and 17 females) from the Wilseyville Valley 13.5 km SE of Ithaca, Tompkins Co., New York, near the village of Brooktondale. This collection was made from a hayfield which had been mowed earlier in the summer. The third collection (Lansing, N.Y.) consisted of 73 individuals (56 males and 17 females) from fields near the Tompkins Co. airport 5.5 km NE of Ithaca. The habitat in this locality consisted of small marshy areas connected by slowly flowing rivulets which run through the fields. The two areas near Ithaca are separated by a linear distance of 16.5 km. In addition to these three main samples, a small sample of eight individuals (4 males and 4 females) was collected in the vicinity of Huntsville, Madison Co., Alabama, on October 3–4, 1973.

Extraction of soluble proteins was accomplished by homogenizing the insects, after removal of legs and wings, in 0.1 ml of a pH 7.0 buffer of 0.1 M tris, 0.001 M EDTA and 5×10^{-5} M NADP. Homogenates were drawn into capillary tubes, centrifuged at 10,000 rpm and stored at –80°C. Techniques of horizontal starch-gel electrophoresis were similar to those of Selander et al. (1971). After electrophoretic separation, the samples were stained for each of five enzymes representing five genetic loci. These were α-glycerophosphate dehydrogenase (α-GPD), phosphoglucomutase (PGM), phosphohexose isomerase (PHI), malate dehydrogenase (MDH) and glutamate-oxaloacetate transaminase (GOT). The first three enzymes (α-GPD, PGM and PHI) are involved in the glycolytic pathway; MDH is a Kreb's cycle enzyme; and GOT represents an important link between the Kreb's cycle and amino acid synthesis. The electrophoretic patterns observed for each of these enzyme-synthesizing loci could clearly be assigned to a genetic model. While PGM is apparently a structural monomer for which heterozygotes give a characteristic two-banded pattern, the remaining four enzymes (α-GPD, PHI, MDH and GOT) appear to be structurally dimeric; the heterozygotes show three bands on electrophoresis (Fig. 1). Gene frequencies were estimated directly from the observed zygotic frequencies.

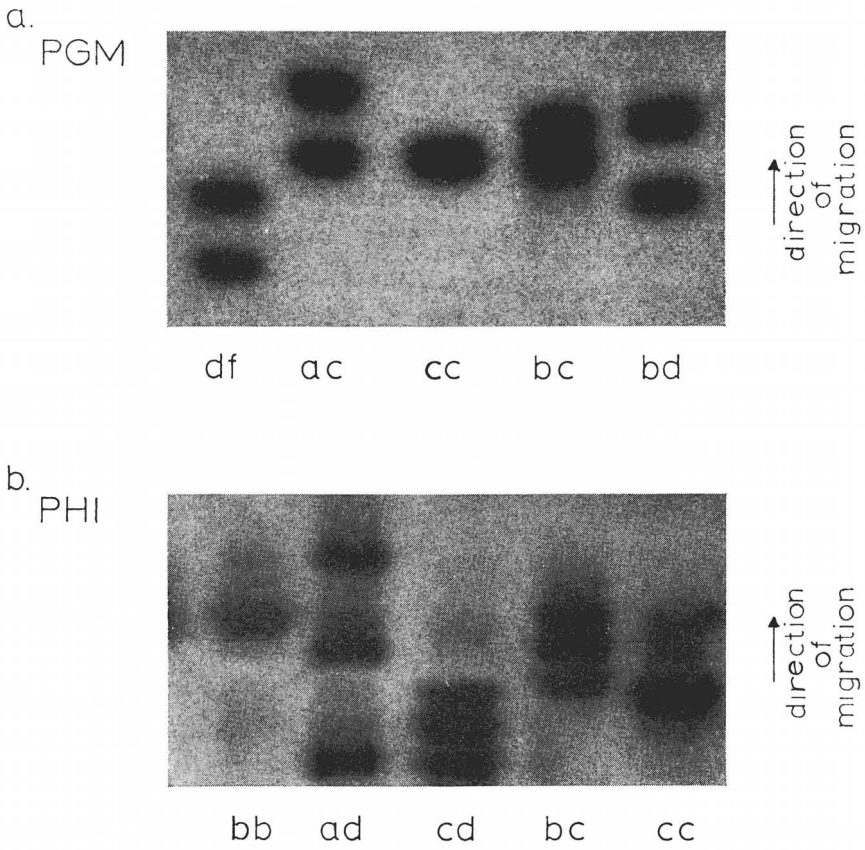

Fig. 1. Typical starch-gels after electrophoresis of *P. tharos* homogenate and staining for PGM, a monomeric enzyme (Fig. 1a) and PHI, a dimeric one (Fig. 1b). Each gel represents five individuals, and presumed genotypes are given below the gels.

RESULTS

Estimated gene frequencies and sample sizes for the five loci examined in *P. tharos* are shown in Table 1. These frequencies were used to calculate expected zygotic frequencies under conditions of Hardy-Weinberg equilibrium using the exact formula of Levene (1949). Deviations of the observed from the expected zygotic frequencies were tested for statistical significance using the G-test for goodness of fit, since for small sample sizes the G-statistic more closely approaches a true Chi-square distribution than does the more traditional χ^2 statistic (Sokal & Rohlf, 1969). Where appropriate, rare alleles were lumped with more common ones for the purpose of statistical analysis. Values for G, degrees of

TABLE 1. Gene frequencies for alleles at five polymorphic loci in *Phyciodes tharos* from four localities, 1973. Alleles are designated alphabetically in order of decreasing anodal mobility. Also included in the table are values for G with appropriate degrees of freedom after lumping G(df) and probabilities (P) associated with the deviations of the observed zygotic frequencies from Hardy-Weinberg expectations.

	Sample size	a	b	c	d	e	f	G(df)	P
PHI									
Silsbee, Texas	87	.03	.30	.49	.18			7.6241(3)	> .05
Lansing, New York	26	.02	.40	.42	.15			2.2002(1)	> .10
Brooktondale, New York	25	.02	.20	.56	.22			5.6162(1)	< .05*
Huntsville, Alabama	8	.06	.38	.31	.25			–	–
GOT									
Silsbee, Texas	87		.99	.01				.0641(1)	> .50
Lansing, New York	59	.01	.97	.02				.2146(1)	> .50
Brooktondale, New York	25	.04	.92	.02	.02			2.9390(1)	> .05
Hunstville, Alabama	7		.86	.14				–	–
α-GPD									
Silsbee, Texas	97		.99	.01				.0641(1)	> .50
Lansing, New York	66	.02	.98					.1918(1)	> .50
Brooktondale, New York	52		1.00					–	–
Huntsville, Alabama	8		.88	.12				–	–
MDH									
Silsbee, Texas	99	.01	.90	.09				.0466(1)	> .50
Lansing, New York	69	.01	.83	.16				.0403(1)	> .50
Brooktondale, New York	55	.02	.86	.12				1.2236(1)	> .10
Huntsville, Alabama	8	.06	.81	.06	.06			–	–
PGM									
Silsbee, Texas	93	.19	.23	.30	.20	.08		13.8875(3)	< .01*
Lansing, New York	69	.09	.20	.36	.33	.02		4.3036(3)	> .10
Brooktondale, New York	54	.08	.21	.34	.31	.03	.03	.4084(3)	> .90
Huntsville, Alabama	8		.25	.38	.38			–	–

* Deviation from Hardy-Weinberg expectation significant at .05 level.

freedom and appropriate probabilities for the observed deviations are also shown in Table 1. Deviations from Hardy-Weinberg equilibrium are statistically insignificant in all but two cases. The general agreement with Hardy-Weinberg expectations exhibited for the other enzyme loci provides evidence for the correctness of the assumed genetic model in each case. The deviations from expected values in the case of PGM from Silsbee, Texas, and that of PHI from Brooktondale, New York, are most probably due to scoring errors, since each of these enzymes is represented by three common allozymes with quite similar mobilities. Alternate interpretations for deviations from Hardy-Weinberg expecta-

tion such as selection or population subdivision seem less likely and are inconsistent with the rest of the data.

The most striking aspect of these results is the geographic constancy of the gene frequencies. As can be seen in Table 1, for the loci examined in this study one finds, with few exceptions, that the rank order of gene frequencies remains the same from population to population; or, that is, that alleles which are the most common in one population are the most common in other populations. In the cases where this is not true, PHI and PGM, variations from this pattern can be readily explained by noting that, in all populations, these loci are represented by 2 or 3 common alleles occurring at similar frequencies, so that alteration of the rank order is produced by small variations of the gene frequencies.

DISCUSSION

The results of the present study indicate that there is little geographic differentiation in allozyme frequencies among populations of *P. tharos*. These results are similar to those obtained by a number of authors working with various species of *Drosophila* (O'Brien & MacIntyre, 1969; Prakash, Lewontin & Hubby, 1969; Rockwood-Sluss, Johnston & Heed, 1969; Ayala, Powell & Dobzhansky, 1971; Ayala et al., 1972) and for the butterfly *Hemiargus isola* (Burns & Johnson, 1971). Such patterns of geographic constancy of gene frequencies are clearly inconsistent with an hypothesis proposing selective neutrality of alleles. Under selective neutrality one would expect to find a random pattern of predominance or fixation of selectively equivalent alleles among geographically separate populations. The existence of a clear pattern, albeit one of clinal variation or geographic constancy, is direct evidence for the action of selection. Kimura & Ohta (1971) argue that, assuming certain conditions concerning effective population sizes and mutation rates, a mobile and widely distributed species may approach panmixis over large portions of its range, thus preventing genetic differentation even among selectively neutral alleles. Although ecological data concerning population densities and migration rates which would allow an assessment of the reasonableness of this approach are not available for *Phyciodes tharos*, other evidence will be introduced later in this discussion which indicates that this species is far from panmictic over the portion of its range we have examined here.

If the observed genetic variability within populations of *P. tharos* is maintained by some form of balancing selection, the patterns observed in the present study should allow some inferences concerning the nature of this selection. In particular, what kind of selection pressures could

lead to a pattern of geographic constancy such as that observed for *P. tharos*? It is possible that, in spite of the large distances involved and the obvious differences in climate between New York, Texas, and Alabama, the collections of *P. tharos* used for this study came from similar microhabitats and that the enzyme loci examined here are subject to similar selection pressures in all populations represented. Although these collections were made in different vegetation types including, for example, hayfields in New York and open pine woods in Texas, it is impossible, with our present information, to exclude the possibility that the convergence of gene frequencies for the various localities is due to factors of the external environment not evident to us, but which remain constant over much of the species' range.

An alternative hypothesis is that the loci examined have been selected to operate in a certain internal physiological and genetic milieu and that the observed pattern of geographic constancy is the result of coadaptation of loci within the species' genome. Prakash, Lewonton & Hubby (1969) proposed such an hypothesis as an explanation for the pattern of genetic constancy observed for populations of *Drosophila pseudoobscura.* Such a pattern need not obtain over all of a species' range nor over the entire species' genome. In a species where gene flow between populations is a rare event, constellations of coadapted alleles could reach frequencies representing independent optima in different populations. However, loci which are thus selected for as integral parts of coadapted gene complexes would not necessarily be affected by external environmental differences. Thus any detectable pattern of geographic variation would not be interpretable in a climatic or microenvironmental context. Moreover, while such internally coadapted genes may be free from variation with climate or habitat, other parts of the genome which interact more directly with the external environment may not be. These genes would be the ones which might show differences that parallel environmental gradients.

This model can explain the apparent discrepancy between our results and those of Oliver (1972). His study of geographic differentiation in four species of Lepidoptera, including *Phyciodes tharos*, entailed comparisons of phenotypic differences in appearance, physiology, and genetic incompatibility between populations from widely separated parts of the species' ranges. He found that variation in phenotypic appearance, physiology and degree of interpopulation compatibility varied discordantly. Clearly, various aspects of population differentiation respond independently to environmental gradients or biogeographic factors; some characteristics may vary geographically while others remain constant.

Epistatic interactions between loci coding for the former type of characteristic and those coding for the latter type would lead to integration of these two parts of the genome within populations, thereby allowing local adaptation. An hypothetical example might help to clarify this point. An allele for a particular enzyme may have identical physiological effects in an individual of a given species regardless of geographic location within the species' range and may, therefore, occur in identical frequencies in all populations, while an allele at another locus coding for the production of this enzyme during the life history of the individual may vary geographically in frequency in a manner closely paralleling climatic differences over the species' range. Crosses between geographically separate populations would demonstrate incompatibility in spite of genic similarity at many loci. In a species such as *P. tharos*, an inhabitant of ephemeral and unstable habitats, selection might well favor an integrated, coadapted genome with high average fitness in a wide range of environments. Local adaptation, then, would involve developmental rates, voltinism and other traits which act as "fine tuning" of the "all-purpose" genotype. Oliver's findings of geographic variation in developmental rates, voltinism and other characters under polygenic control are, therefore, not at odds with our own.

Furthermore, Oliver's results showing geographic differentiation for at least some loci indicate that *P. tharos* is by no means effectively panmictic over large portions of its range. This suggests, as was asserted earlier in this discussión, that the pronounced interpopulation similarities in gene frequencies which we have reported for *P. tharos* are due to selection and not to gene flow.

SUMMARY

Electrophoresis on starch gels was used to separate allelic forms of five enzymes (α-GPD, PGM, PHI, MDH and GOT) in the butterfly *Phyciodes tharos* from 4 localities, 2 in southcentral New York, 1 in Texas and 1 in Alabama. A comparison of gene frequencies between these localities indicated considerable stability—for each enzyme, the various alleles occurred in similar frequencies in all populations. This lack of differentiation suggests that the polymorphisms concerned are maintained by some form of selection and not by random drift of selectively neutral alleles. Since the collections were made over a wide geographic range and in a number of different habitat types, the results of this study suggest that the selective forces involved are probably associated with the maintenance of coadapted gene complexes rather than direct interaction with the environment. It is suggested, however, that local adapta-

tion can still be achieved by the epistatic interaction of these coadapted gene complexes with control genes and modifiers which may be strongly selected by the local environment.

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A SECOND EXTANT COLONY OF *PIERIS VIRGINIENSIS* IN ONTARIO (PIERIDAE)

The relatively recent acceptance of *Pieris virginiensis* Edwards as a species (distinct from *Pieris oleracea*) leads to considerable difficulty in determining its range from existing collections. Local and colonial in its distribution and at the northern limit of its range in Ontario, where it inhabited only certain selected rich hardwoods of the many where *Dentaria* spp. grows, it had been considered extinct in the province. Its previously known stations, cited by Riotte (1967, Proc. Entomol. Soc. Ontario 98: 27–29), at Hamilton, London and Etobicoke, have all succumbed to urban development. However, in 1965 it was rediscovered by Holmes in the Halton County Forest Riotte (1967), and since then this extensive woods has remained the only known Ontario locality despite extensive field work throughout the province.

Unusually interesting, then, was the reported specimen by Warren (1963, Entomol. Ts. 84: 1–4), from "Grand La Cloche Island" (presumably Great Cloche Island, Manitoulin District) in the British Museum, with the implication of a possible surviving colony in that area.

Now, despite examinations of many stations in southern Ontario for the relatively common *Dentaria*, until 1973 *Pieris virginiensis* was found only in one. In the heavily glaciated limestone of Manitoulin District even suitable habitat for *Dentaria* is unusual, namely accumulations of rich soil sufficiently deep to support hardwood forest. To my knowledge, such habitat is absent on Great Cloche Island. Furthermore, even amongst these relatively infrequent hardwood forests, the occurrence of *Dentaria* is infrequent. Soper (1973, pers. comm.), was able to find only four stations, all of *Dentaria diphylla*, in the whole district, only one of which was on Manitoulin Island itself. A survey of Manitoulin Island by the author and some members of the Toronto Entomological Society failed to reveal any additional *Dentaria* in the many possible suitable sites examined until, in May 1972, the author was directed to a badly cut-over maple woods in the central part of the island where moderate numbers of both *Dentaria diphylla* and *laciniata* were in flower. Despite several visits no Pieridae were observed there in 1972. However, on 20 May 1973, four female specimens were taken flying weakly amongst the clearings and along paths in the woods. Identification has been confirmed by J. C. E. Riotte, and two specimens deposited in the collection of the Department of Entomology and Invertebrate Zoology, Royal Ontario Museum.

This collection completes a link in the chain of occurrences of this species between Michigan in the west and southern Ontario and New York to the southeast, raising the hope that still further populations may yet be discovered. *Pieris virginiensis*, because of its habits and local habitat, which is especially vulnerable to urban development and cutting for firewood, must be regarded as an endangered species in Ontario.

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