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THE RELATIONSHIP BETWEEN HETEROZYGOSITY FOR ENZYME LOCI AND DEVELOPMENTAL HOMEOSTASIS IN PERIPHERAL POPULATIONS OF AQUATIC BIVALVES (UNIONIDAE)

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The relationship between heterozygosity for enzyme loci and phenotypic variability remains poorly understood despite continued, if sporadic, interest by evolutionary biologists. Breeders of domestic plants and animals, at least in outcrossing species, have long recognized that the reduction in genetic heterozygosity caused by inbreeding results in a loss of developmental homeostasis and a consequent increase in phenotypic variability (Lerner 1954). Experiments with laboratory populations of *Drosophila* have supplied corroborating, though sometimes contradictory evidence (Soulé 1973), and recently, Mitton (1978) and Eanes (1978) found that heterozygotes for enzyme loci are morphologically less variable than homozygotes for those loci in natural populations of killifish and butterflies. To my knowledge, peripheral populations have not been examined to test the generality of this relationship, despite their potential for information on the correlation between decreased enzyme heterozygosity and morphological variability (Soulé 1973). The results of this study indicate a distinct relationship between decreased genetic heterozygosity and loss of developmental homeostasis (as measured by deviations from bilateral symmetry) for peripheral populations of two freshwater bivalve species.

If developmental homeostasis is defined as the capability of an organism to form an average phenotype in the face of external perturbations (Schmalhausen 1949, Lerner 1954), then small deviations from bilateral symmetry should constitute a reasonable measure of a decrease in homeostasis (Soulé 1967, 1979). It is important to point out, however, that the character measured should not be under strong stabilizing selection, otherwise phenodeviants might not persist. For this reason, minor right-left differences in the number of plicae on the labial palps (ridged, ciliated structures involved in sorting and transferring food to the mouth) were chosen to measure bilateral asymmetry in this study.

These data form a subset of a larger study on the genetic and morphological variability of unionid bivalves in Nova Scotia, Canada. Present geographical distributions of these freshwater bivalves are still strongly influenced by Wisconsin glaciation: Recolonization of previously glaciated areas from southern refuges

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FIG. 1.—Location of the sample site indicates a possible route of immigrat

such as Virginia has been slow, and richness with increasing distance fr Nova Scotia, this pattern manifes richness with distance from the l Clarke 1961; Clarke and Rick 196

From a data set of 12 enzyme sy heterozygotes (leucine aminopep glucomutase (*pgm* I and II), and g *omplanata; lap, pgm* I and II, *Lampsilis radiata*) were chosen. similar to those of Ayala et al. (197 y Davis et al. (1981). Twenty in *lliptio* and five populations of *Lam* he electrophoretic study. The resu outer labial palps were excised fro formalin) individuals and placed u each palp (usually 80–90 plicae/palp 40×. Ten to 15 individuals were mmetry; the results are presented heterozygosity and bilateral asym ndication that asymmetry is directi plicae than the other.

Average heterozygosity for the fi hat of conspecific southern popu

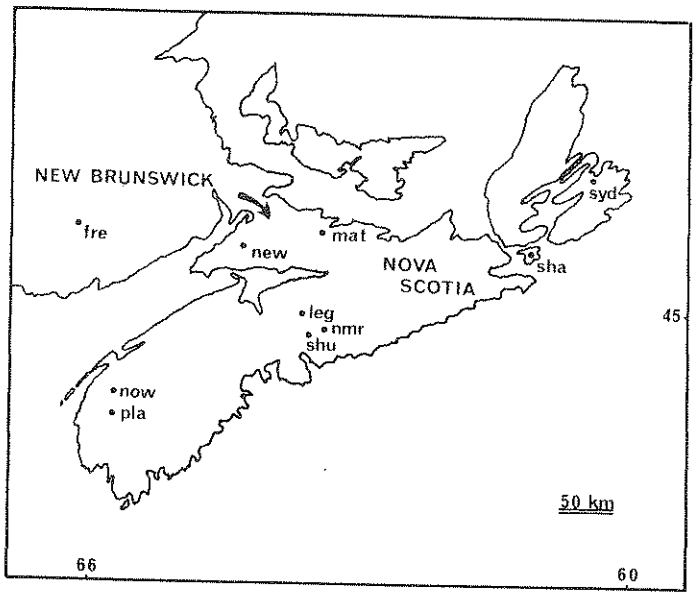

FIG. 1.—Location of the sample sites in Nova Scotia and New Brunswick, Canada. Arrow indicates a possible route of immigration across the Isthmus of Chignecto.

uch as Virginia has been slow, and is reflected in a geometric decrease in species chness with increasing distance from source areas (Sepkoski and Rex 1974). On Nova Scotia, this pattern manifests itself in a progressive decrease in species chness with distance from the land bridge to New Brunswick (Athearn and Clarke 1961; Clarke and Rick 1963; fig. 1).

From a data set of 12 enzyme systems, five loci with the highest proportion of eterozygotes (leucine aminopeptidase (*lap*), hexokinase (*hex*), phospho-lucomutase (*pgm* I and II), and glucose phosphate isomerase (*gpi*) for *Elliptio omplanata; lap, pgm* I and II, *hex*, and malate dehydrogenase (*mdh* I) for *Lampsilis radiata*) were chosen. Allelic frequencies were scored by methods imilar to those of Ayala et al. (1973); the electrophoretic methods are described y Davis et al. (1981). Twenty individuals from each of eight populations of *Elliptio* and five populations of *Lampsilis* collected in similar habitats were used in he electrophoretic study. The results are presented in table 1. The right and left outer labial palps were excised from relaxed (sodium nembutal) and fixed (10% formalin) individuals and placed under a cover glass. The number of plicae on each palp (usually 80–90 plicae/palp) were then counted under a magnification of 40×. Ten to 15 individuals were used to determine deviations from bilateral symmetry; the results are presented in table 2. The relationship between average heterozygosity and bilateral asymmetry is presented in figure 2. There is no indication that asymmetry is directional; one side did not consistently have more plicae than the other.

Average heterozygosity for the five loci of Nova Scotian *Elliptio* is lower than that of conspecific southern populations (*H* for populations from Delaware,

TABLE 1

ALLELE FREQUENCIES AND OBSERVED HETEROZYGOSITIES (H) FOR POPULATIONS OF *Elliptio complanata* AND *Lampsilis radiata*

LOCUS	ALLELE	*Elliptio complanata* POPULATIONS							
		MAT	SHU	NEW	PLA	SHA	NMR	SYD	NOW
GPI	14	.08	.25	.08	.31	.21		.13	.30
	8	.92	.75	.92	.69	.79	.95	.83	.70
	2						.05	.04	
PGM I	H	.08	.15	.03	.20	.10	.05	.13	.15
	18	.14	.05				.08		
	15	.86	.93	.84	.96	.13	.30	.65	.90
	12		.02	.16	.04	.75	.50	.35	.10
	9					.13	.13		
PGM II	H	.12	.08	.08	.04	.10	.30	.20	.10
	34	.05	.05	.10		.25			.03
	32	.05		.13		.05	.05		
	30	.66	.78	.43	.93	.65	.68	.85	.89
	28	.08	.05	.20	.08	.05	.10	.15	.08
	26	.16	.10	.15			.17		
LAP	H	.15	.13	.40	.15	.30	.28	.10	.05
	42			.03		.20			
	40			.06					.05
	38	.47	.20	.08	.04		.05	.15	
	36	.18	.23	.14	.04	.43	.10		.50
	34	.05		.14	.14		.13	.08	.25
	32	.24	.43	.39	.64	.37	.43	.67	.20
	30	.05	.10	.08	.14		.02		
	28		.04	.08			.13	.10	
	26						.10		
	23						.05		
	11								
HEX	37						.03		
	34						.65		
	31	.40	.22	.36	.26	.15	.15	.32	.10
	28	.60	.78	.64	.74	.85			

	H	.39	.40	.25	.21	.27	.35	.15	.27
	34						.65		
	31	.40	.22	.36	.26	.15	.15	.32	.10
	28	.60	.78	.64	.74	.85		.68	.90
	H	.13	.13	.11	.11	.00	.20	.17	.00

Locus	Allele	*Lampsilis radiata* Populations				
		NEW	FRE	SHU	LEG	MAT
PGM I	18		.04		.05	.32
	15	1.0	.87	1.0	.95	.63
	13		.09			.05
	H	.00	.12	.00	.05	.25
PGM II	28	1.0	.94	1.0	1.0	.95
	26		.06			.05
	H	.00	.06	.00	.00	.05
LAP	34	.47	.04			.03
	32	.53	.75	.92	1.0	.67
	30		.21	.08		.30
	H	.30	.21	.08	.00	.22
HEX	35		.06			
	32	1.0	.94	1.0	1.0	1.0
	H	.00	.06	.00	.00	.00
MDH	19		.13			
	15	1.0	.87	1.0	1.0	1.0
	H	.00	.13	.00	.00	.00

Note.—For location of the collection localities, refer to figure 1.

TABLE 2

AVERAGE ASYMMETRY (A) (| No. plicae right–No. plicae left |) AND AVERAGE HETEROZYGOSITY (H) (observed) FOR CANADIAN *Elliptio complanata* AND *Lampsilis radiata* (In Addition, fluctuating asymmetry of the number of inhalent siphon papiliae (ASP) is presented for *L. radiata*.

	Elliptio complanata POPULATIONS							
	MAT	SHU	NEW	PLA	SHA	NMR	SYD	NOR
A	.23	.50	.34	2.14	1.66	.10	1.00	4.0
H	.181	.178	.174	.142	.154	.236	.150	.13

	Lampsilis radiata POPULATIONS				
	FRE	SHU	LEG	MAT	NEW
A	.90	4.00	3.25	1.75	2.30
H	.116	.016	.006	.104	.060
ASP ...	2.34	6.53	8.67	2.12	4.83

NOTE.—Refer to figure 1 for location of populations.

Maryland, and New Jersey = 0.34 ± 0.03; Davis et al. 1981), and tends to decrease further with increasing distance from New Brunswick. Reduction in heterozygosity in these peripheral populations is brought about mainly by loss of alternative alleles. Heterozygosity values for southern populations of *Lampsilis* are not available; trends within Nova Scotia are consistent with those of *Elliptio*.

Both the standard deviation and the average of deviations from labial palp bilateral symmetry increased in populations with increasing levels of homozygosity. Deviations in the relatively heterozygous populations were generally on the order of 0–2 plicae/individual; in more homozygous populations this measure

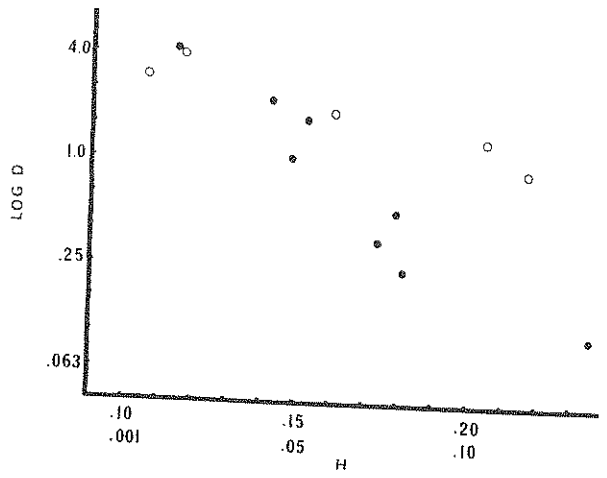

FIG. 2.—Relationship between average heterozygosity (H) for five enzyme loci and deviations from bilateral symmetry (D) for *Elliptio complanata* (solid circles, upper scale: $P << .01$, $r^2 = .897$) and *Lampsilis radiata* (open circles, lower scale: $P << .01$, $r^2 = .923$).

increased to 1–10 plicae per individual ($P < .01$; t-test of means of heterozygous vs. homozygous populations corrected for unequal variances). There also is some evidence that deviations from symmetry in the number of palp plicae are indicative of a more generalized pattern of deviation from a normal phenotype; such deviations manifest themselves in patterns of valve dentition (loss or addition of lateral teeth in more homozygous populations), and fluctuating asymmetry of siphonal papillae (table 2). Soulé (1967, 1979) found a similar correlation among bilateral characters in insular lizard populations.

It does not appear as if there is any consistent relationship between heterozygosity for any particular locus and asymmetry values for these species, contrary to the observations of Mitton (1978), who could divide a population into two distinct groups of different morphological variability on the basis of a single locus. Average heterozygosity over a number of loci seems the strongest predictor of deviation from bilateral symmetry in this study. (Note, however, that there exist some significant differences between the methodology of Mitton and that employed here. Asymmetry could not be accurately determined except by using preserved specimens, which are obviously unsuitable for subsequent electrophoretic analysis. Asymmetry values associated with individual heterozygosities could not be determined, and have to be approximated here by averaging asymmetries and heterozygosities over a population. While such averaging can lead to spurious results, the degree of difference in asymmetry between homozygous and heterozygous populations as well as the consistency of the trend for the two species examined constitute evidence that the method is valid; lack of precision should be outweighed by the value of working with natural populations.) It appears that similar values of asymmetry correspond to much lower values of heterozygosity for *Lampsilis* than *Elliptio* (fig. 2). The former is generally much more monomorphic for enzyme loci than the latter; levels of heterozygosity which determine homeostasis can thus be different from species to species.

There are several indications that environmental stress can result in significant deviations from bilateral symmetry in several organisms (Thoday 1956; Valentine and Soulé 1973; Valentine et al. 1973). Since the individuals chosen for this study were sampled from peripheral populations, it could be hypothesized that environmental stress is responsible for the asymmetries rather than homozygosity. This explanation is unlikely for three reasons. First, the populations of both *Elliptio complanata* and *Lampsilis radiata* are composed of a large number of individuals, some of which attain sizes equal to the largest individuals observed in other parts of the geographic ranges. Growth rates (as measured by spacing between annual rings), however, are slow, which is not surprising considering the short growing season at these northern latitudes. Large individuals could be in excess of 30 yr old; the average age of individuals for which asymmetry values were calculated (estimated by the number of annual growth rings) is in excess of 16 yr. Second, there is no evidence of any reproductive stress in these populations; gametogenesis is normal, and gravid females carry numbers of larvae no different from those observed in other parts of the geographic range (personal observation). Similar reproductive data was used to determine reproductive

maladaptedness and hence environmental stress of a marginal population of an other bivalve (Kat 1982). Third, stressed populations are often characterized by drastic fluctuations in population numbers (e.g., Welch 1968; Gallagher and Wells 1969; Kat 1982). Such fluctuations were not observed for these populations densities in 1980 and 1981 were no different than those estimated by Athearn and Clarke (1961) and Clarke and Rick (1963).

While the results of this study are important in determining the generality of the relationship between electrophoretically determined heterozygosity for enzyme loci and developmental homeostasis (however, see Thoday 1958), they are as inconclusive as previous studies in determining the source of this effect (Eanes 1978; Mitton 1978). In fact, this study contains the same paradox as earlier studies: The relationship between heterozygosity of the 14 loci examined and the rest of the genome is unknown. As Eanes (1978) points out, it is unreasonable to extrapolate heterozygosity values for a small subset of all possible loci to the entire genome. The specific contribution of any locus is bound to be small: most genes are known to be pleiotrophic, and most characters affected by many genes (Speiss 1977; Futuyma 1979). Nevertheless, in this particular study it might be possible to make a stronger case for a correlation between the loci surveyed and the rest of the genome. Peripheral populations (in species other than those of *Drosophila*) can exhibit loss of genetic polymorphisms resulting from such factors as inbreeding among founders, reduced immigration, and genetic drift following bottlenecks (Soulé 1973; Nei et al. 1975). Such allelic depauperization occurs among the loci surveyed for the Nova Scotian populations, and while the sample is small, trends among these loci could thus be reasonable indicators of total heterozygosity of the genome, assuming that all loci are affected equally by founder effects.

Lerner (1954) proposed that loss of developmental homeostasis and the reduction of associated canalization creates the potential for establishment of new balanced genotypes. In this case, differences in the locally prevailing selective regimes of the peripheral populations could result in formation of such new genotypes which might not combine well with ancestral genotypes, thereby creating the potential for species-level differentiation. This scenario places a slightly different emphasis on the effects of founder events on species formation than that currently accepted (*sensu* Mayr 1942) in that it stresses the importance of homozygosity of founder populations in novel environments in addition to changes in gene frequencies which follow such founder events.

SUMMARY

The results of this study indicate a distinct relationship between heterozygosity for enzyme loci and phenotypic variability; peripheral populations of two freshwater bivalve species from Nova Scotia, Canada, exhibit an increase in deviations from bilateral symmetry with a decrease in heterozygosity. Average heterozygosity over five polymorphic loci seems the strongest predictor of deviation from bilateral symmetry. Levels of heterozygosity which determine developmental homeostasis are different for the two species examined. Levels of heterozygosity

detected electrophoretically are proposed to be indicative of overall levels of heterozygosity of the genome in these peripheral populations. Allelic depauperization detected electrophoretically is hypothesized to be due mainly to founder events, which are proposed to affect all susceptible loci equally.

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