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Biochemical Correlates of Genetic Variation in Marine Lower Invertebrates

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In this paper extensive data on enzyme variation in 23 species of coelenterates and sponges were used to investigate the possible correlation of levels of genetic variation with various parameters of enzyme molecular structure and function. The data provide an opportunity not only to look for such correlations for the first time in lower invertebrates, but also to study organisms with far higher average levels of genetic variability than those used in any previous work. A clear inverse relationship was found between enzyme subunit number and levels of polymorphism, with monomers being more variable than dimers or tetramers. No significant difference in polymorphism could be found in enzymes of the functional groups I and II of Gillespie and Langley (1974). Regulatory enzymes appeared to be significantly more polymorphic than nonregulatory enzymes, but a significant relationship was observed between regulatory power and subunit structure which could bias this result. The results suggest that both neutralist and selectionist ideas may have a useful role to play in the understanding of the factors which can influence or limit levels of genetic variation.

KEY WORDS: heterozygosity; neutralism; selectionism; Porifera; Coelenterata; enzyme structure; enzyme function.

INTRODUCTION

A great volume of work on the genetic variation of animal populations has been published in the past 20 years (reviews by, e.g., Nevo, 1978, 1983;

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Kimura, 1983). Two major conclusions may be drawn from this bulk of data: (1) between different species there can be large differences in levels of genetic variability (heterozygosity); and (2) some enzymes are apparently more likely to be polymorphic than others. The first fact has been tentatively explained either in terms of drift and stochastic phenomena (reviewed by Kimura, 1983) or in terms of selection (reviewed by Hartl, 1980; Nevo, 1983). Similarly, the interenzyme differences in heterozygosity have been attributed to differences in either structural parameters, for example, subunit number (Zouros, 1975, 1976; Ward, 1977; Koehn and Eanes, 1978), size (Harris *et al.*, 1977; Koehn and Eanes, 1977; Nei *et al.*, 1978; Ward, 1978), subcellular location (Ward and Skibinski, 1988), or physiological role (Gillespie and Kojima, 1968; Kojima *et al.*, 1970; Johnson, 1971, 1973, 1974, 1976; Gillespie and Langley, 1974; Powell, 1975; Ward, 1977; Gojobori, 1982). The vast bulk of data used in such work, however, is derived from studies of vertebrates or of *Drosophila*. Marine invertebrates with (generally) higher levels of genetic variation have been largely omitted from such studies, reflecting perhaps the shortage of available information. In the present work data are presented showing levels of enzyme variation for 23 species of marine coelenterates and sponges, groups for which such data were previously scarce. These animals show generally very high levels of genetic variability, a feature which is itself of note. An analysis is then carried out of levels of variation in relation to enzyme molecular parameters of structure and function for types of organisms which not only have been excluded from similar earlier studies, but also show a far higher mean level of polymorphism than the higher animals used previously.

MATERIALS AND METHODS

Collection sites and measures of levels of genetic variation for each species studied are given in Table I. Loci were considered polymorphic when the frequency of the most common allele was less than 0.95. Electrophoretic and staining techniques followed standard procedures (Brewer, 1970; Harris and Hopkinson, 1978). Enzyme nomenclature follows Harris and Hopkinson (1978). All samples were analyzed in the same laboratory, under the same conditions, and were interpreted in the same way. This approach should eliminate possible errors due to interlaboratory variation in heterozygosity estimates (Smith and Fujio, 1982).

The subunit numbers of the enzymes studied were estimated from heterozygote banding patterns and generally corresponded to known subunit structures in other organisms (see, e.g., Hopkinson *et al.*, 1976; Ward, 1977). Information on enzyme function was obtained from Johnson (1974), Lehninger (1975), and Gojobori (1982).

Table I. Parameters of Genetic Variation for 23 Species of Marine Sponges and Coelenterates*

Species	Taxon	Location	Depth	n_l	n_i	He	$P_{0.95}$
Actinia equina	Hexacorallia	Isle of Man	0	18	22	0.260	0.788
Actinia prasina	Hexacorallia	Isle of Man	0	18	19	0.250	0.611
Actinia sp.	Hexacorallia	Isle of Man	0	18	14	0.198	0.411
Actinothoe sphyrodeta	Hexacorallia	Isle of Man	20	14	23	0.169	0.429
Adamsia carciniopados	Hexacorallia	Isle of Man	30	16	19	0.249	0.750
Agelas oroides	Demospongiae	Mediterranean	15	18	8	0.215	0.611
Anemonia viridis	Hexacorallia	Isle of Man	10	22	17	0.139	0.455
Axinella damicornis	Demospongiae	Mediterranean	15	8	7	0.087	0.250
Axinella polypoides	Demospongiae	Mediterranean	15	8	7	0.125	0.250
Cassiopeia andromeda	Scyphozoa	Red Sea	12	5	10	0.106	0.600
Chondrilla nucula	Demospongiae	Mediterranean	15	16	8	0.187	0.563
Chondrosia reniformis	Demospongiae	Mediterranean	15	12	8	0.335	0.833
Halichondria panicea	Demospongiae	Isle of Man	0	15	18	0.234	0.688
Metridium senile	Hexacorallia	Isle of Man	10	19	9	0.200	0.526
Mycale macilenta	Demospongiae	Isle of Man	40	18	7	0.189	0.500
Petrosia ficiformis	Demospongiae	Mediterranean	15	10	8	0.312	0.700
Sarcodictyon roseum	Octocorallia	Isle of Man	40	13	12	0.199	0.462
Sarcophyton ehrenbergi	Octocorallia	Red Sea	14	13	14	0.254	0.615
Suberites luridus	Demospongiae	Isle of Man	40	18	13	0.195	0.611
Suberites pagurorum	Demospongiae	Isle of Man	40	16	16	0.335	0.750
Suberites rubrus	Demospongiae	Isle of Man	40	18	12	0.167	0.667
Urticina eques	Hexacorallia	North Sea	80	18	25	0.401	0.833
Urticina felina	Hexacorallia	Isle of Man	5	18	35	0.363	0.833

*Depth of collection site in meters below chart datum. n_l—number of loci studied; n_i—number of individuals analyzed; He—mean heterozygosity per locus; $P_{0.95}$—proportion of loci polymorphic.

RESULTS

Over all species studied, levels of polymorphism (expressed as the proportion of loci polymorphic) varied between 0.250 and 0.833, and the mean heterozygosity per locus (He) varied between 0.087 and 0.401 (Table I). Over the various enzymes used levels of polymorphism varied between 0.25 and 1.00 (mean, 0.639), and mean heterozygosity values ranged from 0.07 to 0.43 (mean, 0.225) (Tables II and III).

The subunit structure of the enzymes analyzed in coelenterates and sponges (as deduced from banding patterns) was found to be generally the same as those already known in vertebrates. Although not surprising, this indicates a notable conservatism in tertiary structure along evolutionary lines. Malate dehydrogenase has been reported as a monomer in one of the sea anemone species used ([*Actinia equina* (Quicke and Brace, 1983)], but banding patterns for this species were observed in this study as being typical for dimeric enzymes (three-banded heterozygotes), as also found in other sea

Table II. Enzyme Commission Number (EC No.), Subunit Structure (Subunits), Glucose Metabolism (Gluc), Regulatory Power (Reg), Number of Monomorphic (Mono), Polymorphic (Poly), and Total (All) Loci, Proportion of Loci Polymorphic (Poly), and Observed Mean Heterozygosity (H) for Each of the 22 Enzymes Studied[a]

					Loci			
Enzyme	EC No.	Subunits	Gluc	Reg	Mono	Poly	All	Poly	H
Acon	4.2.1.3	1	−	?	0	7	7	1.00	0.43
Ada	3.5.4.4	1	−	+	1	5	6	0.83	0.33
Aldox	1.2.3.1	2	−	+	4	5	9	0.56	0.12
Cat	1.11.1.6	4	−	−	3	7	10	0.70	0.22
Est	3.1.1.1	1	−	?	11	30	41	0.73	0.28
Fum	4.2.1.2	4	+	−	5	4	9	0.44	0.07
Gdh	1.4.1.3	1	+	+	2	6	8	0.75	0.18
Got	2.6.1.1	2	+	−	11	10	21	0.48	0.16
Gpd	1.1.1.8	2	+	−	2	5	7	0.71	0.24
Hk	2.7.1.1	1	+	+	5	21	26	0.81	0.31
Idh	1.1.1.42	2	+	−	1	4	5	0.80	0.20
Lap	3.4.1.1	1	−	−	4	8	12	0.67	0.29
Mdh	1.1.1.37	2	+	−	12	11	23	0.48	0.18
Me	1.1.1.40	4	+	+	3	8	11	0.73	0.27
Mpi	5.3.1.8	1	+	+	2	10	12	0.83	0.34
Odh	1.1.1.1	2	−	−	2	5	7	0.71	0.25
Pep	3.4.11.1	2	−	−	15	14	29	0.48	0.14
Pgd	1.1.4.4	2	+	−	8	14	22	0.64	0.21
Pgi	5.3.1.9	2	+	+	9	19	28	0.68	0.21
Pgm	2.7.5.1	1	+	+	4	18	22	0.82	0.36
Sod	1.15.1.1	4	−	−	15	5	20	0.25	0.08
Xod	1.2.3.1	2	−	+	7	7	14	0.50	0.16
All					126	223	349	0.64	0.23

[a]Enzymes analyzed for fewer than five loci have been excluded.

anemones of the same genus (Ayre, 1984) and in vertebrates (Hopkinson *et al.*, 1976). Glutamate dehydrogenase appeared to show two-banded heterozygotes and thus is classed here as a monomer, although thought to consist of six subunits in mammals (Dixon and Webb, 1979).

Enzyme Subunit Structure and Molecular Weight

There is an apparent relationship between enzyme subunit number and level of polymorphism over the range of species studied (Table IV). Monomeric enzymes are far more polymorphic than dimeric enzymes ($\chi^2 = 12.48$; df = 1; $P < 4 \times 10^{-4}$), and dimeric enzymes are more polymorphic than tetrameric enzymes, although the difference is not statistically significant ($\chi^2 = 1.25$; df = 1; $P = 0.26$). These conclusions are similar to those of Ward (1977), who studied data from a wide range of vertebrate and some invertebrate species

Table III. Number of Monomorphic (Mono), Polymorphic (Poly), and Total (All) Loci, Proportion of Loci Polymorphic (Poly), and Observed Mean Heterozygosity (H) for Enzymes of Various Structural or Functional Types (for Details See Text)*

Enzyme type	Loci			Poly	H
	Mono	Poly	All		
Monomeric	29	105	134	0.78	0.290
Dimeric	71	94	165	0.57	0.181
Tetrameric	26	24	50	0.48	0.148
Glucose metabolizing	64	130	194	0.67	0.236
Non-glucose metabolizing	62	93	155	0.60	0.212
Regulatory	37	99	136	0.73	0.262
Nonregulatory	78	87	165	0.53	0.172
All	126	223	349	0.64	0.225

*Enzymes analyzed for fewer than five loci have been excluded.

(see also Koehn and Eanes, 1978). The reason for this apparent link between subunit number and level of genetic variation is not immediately obvious. One suggestion put forward by previous authors is that new mutations would more frequently be less deleterious (more neutral) in monomeric enzymes than in multimeric ones. This is to be expected because of the necessity of maintaining spatial compatibility between the subunits of the latter. Monomeric enzymes would thus have greater freedom to mutate (that is, to mutate and survive in the population) than dimeric or tetrameric enzymes and would, consequently, evolve faster (Dickenson, 1971; Kimura and Ohta, 1974). Similarly, dimeric molecules would be subject to rather fewer restrictions than tetramers.

A relationship also between subunit size and level of genetic variation has been found for *Drosophila* (Koehn and Eanes, 1977) and for vertebrates (Ward, 1978; see also Nei *et al.*, 1978), although not in a study of extensive

Table IV. Contingency Chi-Square Values, Significances, and Degrees of Freedom for the Effects of Structural and Functional Parameters on Levels of Polymorphism

Variable 1	Variable 2	Enzyme loci	N	χ^2	df	P
Subunit number	Polymorphism	All	349	21.06	1	0.00003
Glucose metabolism	Polymorphism	All	349	1.8[illegible]	1	0.18
Regulatory power	Polymorphism	All	301	12.72	1	0.004
Regulatory power	Subunit number	All	20	—	—	0.03*
Regulatory power	Polymorphism	Monomeric	86	0.60	1	0.44
Regulatory power	Polymorphism	Multimeric	215	2.96	1	0.09
Subunit number	Polymorphism	Regulatory	136	5.62	1	0.02
Subunit number	Polymorphism	Nonregulatory	165	1.06	1	0.30

*Fisher's exact test was used in this case, because of the small size of each class.

data for humans (Harris *et al.*, 1977). Such a relationship may be predicted from the "neutralist theory" of molecular evolution (Kimura, 1968, 1983), because the expected mutation rate will be greater in larger molecules (Nei *et al.*, 1978). Gojobori (1982) classified different enzymes into three arbitrary groups, according to levels of genetic variation, and observed that the average molecular weights (based on estimates from humans) were 77,200, 52,700, and 41,600 for the high-, medium-, and low-heterozygosity groups, respectively. The results for coelenterates and sponges cannot be analyzed in terms of subunit molecular weight because estimates of this parameter are not available for the species used. The molecular weights of homologous glycolytic enzymes in different taxonomic groups may be generally quite similar (Ruth and Wold, 1976), but it cannot validly be assumed that sponges and sea anemones have enzyme subunit sizes similar to, for example, those of humans.

Metabolism

It has been suggested (Gillespie and Kojima, 1968; Kojima *et al.*, 1970) that loci coding for enzymes catalyzing reactions related to glucose metabolism (glycolysis, the pentose shunt, and the Krebs cycle) should show a lower level of genetic variation than loci which code for enzymes in less specific metabolic pathways. This hypothesis was later presented in a slightly modified and more general form by Gillespie and Langley (1974), who classified enzymes into types I (enzymes in secondary metabolism, not substrate specific) and II (enzymes within major metabolic pathways, substrate specific). This hypothesis has been used by Nelson and Hedgecock (1980) to explain variations in levels of genetic variation in decapod crustacean species.

A significant difference in heterozygosity levels has been found between glucose- and non-glucose-metabolizing enzymes for invertebrate species (Ward, 1977), but this difference was not found in vertebrate species (Ward, 1977). However, a significant relationship was also found by Gojobori (1982) surveying data for vertebrates and *Drosophila* spp. In a large survey of data from teleost fish, no significant difference was found in levels of polymorphism in enzymes from groups I and II (Smith and Fujio, 1982).

Our data for coelenterates and sponges show no apparent association between heterozygosity levels and enzyme group (Table IV). Of the three most polymorphic enzymes, two (Pgm and Hk) are glucose metabolizing and are likely to be subject to a series of "functional constraints" such as substrate specificity and regulatory function (Johnson, 1974; Gojobori, 1982). The results for these organisms, thus, do not confirm the conclusion of Gojobori (1982).

It has also been proposed that the regulatory power of the enzyme may play a more important role than its relative position in the central or

peripheral metabolism (Johnson, 1973, 1974, 1976). Regulatory enzymes are important because they "set the pace" of metabolism, regulating direction and volume of flow along metabolic pathways (Lehninger, 1975; Hochachka and Somero, 1984). These enzymes are probably more sensitive to selection than the nonregulatory ones (Johnson, 1971). If that is the case, then selectionist and neutralist predictions will be totally different for this group; neutralists will expect these enzymes to be less variable, because of higher functional constraints (Gojobori, 1982), whereas selectionists will predict greater variability in these enzymes, as a means of adaptation to changing environments (Johnson, 1974). The regulatory enzymes of coelenterates and sponges are significantly more polymorphic than the nonregulatory ones (Table IV), seemingly contradicting neutralist expectations. However, knowledge of the biochemistry and ecology of coelenterates and sponges is sparse (see, e.g., Bergquist, 1978; Hochachka and Somero, 1984) and, therefore, any suggestions of a relationship between function and genetic variation in these groups must be made with caution (see, e.g., Selander, 1976).

DISCUSSION

As previously observed for more "complex" animals (Johnson, 1976; Harris *et al.*, 1977; Koehn and Eanes, 1977, 1978; Ward, 1977, 1978; Nei *et al.*, 1978) there seems to exist in coelenterates and sponges a relationship between levels of enzyme polymorphism and certain functional or structural parameters of the enzyme molecules. Of the various parameters which have been hypothesized to influence interlocus differences in levels of polymorphism (see Table V), subunit number and regulatory power appear to be those most highly correlated with the observed levels of polymorphism of the enzyme loci of the organisms studied here.

Table V. Predictions of Different Hypotheses as to the Levels of Polymorphism at Different Enzyme Loci

Hypothesis	High polymorphism	Low polymorphism	Ref.[a]
Heterotic advantage	Multimeric	Monomeric	1
Subunit structure	Monomeric	Multimeric	2, 3, 4
Subunit size	Large	Small	4, 5, 6
Glucose metabolism	Nonglucose	Glucose	7
Substrate origin	Exogenous	Endogenous	8
Metabolic position	Peripheral	Central	8, 9
Metabolic position	Peripheral	Central, regulatory	10
Regulatory power	Regulatory	Nonregulatory	11, 12

[a]References: (1) Fincham, 1972; (2) Zouros, 1975; (3) Ward, 1977; (4) Harris *et al.*, 1977; (5) Koehn and Eanes, 1977; (6) Ward, 1978; (7) Gillespie and Kojima, 1968; (8) Johnson, 1973; (9) Gillespie and Langley, 1974; (10) Gojobori, 1982; (11) Johnson, 1974; (12) Johnson, 1976.

However, it should be noted that there is always a possibility that with unequal numbers of loci studied, certain enzymes could bias the overall results for a particular functional or structural group or groups of enzymes. For example, it could be argued that since about 31% of the monomeric loci studied here were esterases (see Table II), these may have biased the sample and caused a spuriously significant result for monomers as a whole. In the case of this particular example, since esterases appear to be on average less variable than other monomeric loci, they alone are unlikely to have caused monomers to appear more variable than other enzymes. Without further data it is generally difficult to come to any conclusions as to the possible effects of such sources of potential bias.

The two hypotheses that genetic variability is correlated with subunit structure or with regulatory power are not mutually exclusive: levels of genetic variation may be limited by structural constrains (such as subunit number) but may, at the same time, be influenced by increased selection acting on key (regulatory) enzymes in metabolism. A major factor to be taken into account, however, is that these two variables are themselves intercorrelated. Most of the nonregulatory enzymes are multimeric, whereas monomeric enzymes account for more than half of the regulatory enzymes studied (Table II). It is possible, therefore, that the significant relationship found between the subunit structure (or regulatory function) and the level of genetic polymorphism was actually a consequence of the links between these factors (Tables III and V). Indeed, the effect of regulatory power on polymorphism was no longer observed when monomeric and multimeric enzymes were analyzed separately (Table IV). On the other hand, subunit structure was highly associated with levels of genetic variation within regulatory enzymes considered alone but not in nonregulatory enzymes alone (Table VI). The latter results provide an indication that, although regulatory power *per se* may not explain the variation in levels of genetic polymorphism between different enzymes, it may influence, in a synergistic way, the susceptibility of different enzymes to selective pressures. It may be suggested, for example, that mutations at loci coding for multimeric enzymes will more readily be "noticed" by natural

Table VI. Spearman Rank Correlation (Below Diagonal) and Respective Significance (Above Diagonal) for Pairwise Comparisons of Structural and Functional Characteristics

	Subunit structure	Regulatory power	Glucose metabolism	Heterozygosity
Subunit structure	—	0.02*	0.04	0.03*
Regulatory power	−0.53	—	0.59	0.11
Glucose metabolism	−0.02	0.12	—	0.46
Heterozygosity	−0.51	0.37	0.17	—

*Significant correlation.

selection when they occur in regulatory rather than in nonregulatory enzymes.

We can, thus, speculate that factors influencing the amount of genetic variation at any given locus contribute to a dynamic equilibrium, where (1) there is an overall tendency for regulatory enzymes to be more variable than nonregulatory enzymes (as might be expected from "selectionist" predictions) and (2) the structural constraints on enzyme molecules are emphasized in regulatory enzymes, thus making them more prone to stabilizing selection (predictable from "neutralist" arguments). It seems that these apparently opposing selective pressures—one toward heterozygosity in physiologically important enzymes and the other toward homozygosity in structurally constrained molecules—may to some extent be balancing each other, with maximum genetic variation being expected among monomeric regulatory enzymes and minimum in the multimeric nonregulatory group of enzymes.

Clearly the putative interaction between structure and function needs to be studied further, using a larger number and variety of enzyme loci. Such work might help to shed light upon important questions such as why so many regulatory enzymes are monomeric, and it should also give more precise information as to the relative importance of both structural and functional parameters as influences upon the level of genetic variation found in particular molecules.

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REFERENCES

Ayre, D. J. (1984). The effects of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. *Oecologia* **62**:222.

Bergquist, P. R. (1978). *Sponges*, Hutchinson, London.

Brewer, G. J. (1970). *An Introduction to Isozyme Techniques*. Academic Press, New York.

Dickenson, R. E. (1971). The structure of cytochrome c and the rates of molecular evolution. *J. Mol. Evol.* **1**:26.

Dixon, M., and Webb, E. C. (1979). *Enzymes*, 3rd ed., Longman, London.

Fincham, J. R. S. (1972). Heterozygous advantage as a likely general basis for enzyme polymorphisms. *Heredity* **28**:387.

Gillespie, J. H., and Kojima, K. (1968). The degree of polymorphism in enzymes involved in energy production compared to that in non-specific enzymes in two *Drosophila ananassae* populations. *Genetics* **61**:837.

Gillespie, J. H., and Langley, C. H. (1974). A general model to account for enzyme variation in natural populations. *Genetics* **76**:837.

Gojobori, T. (1982). Means and variances of heterozygosity and protein function. In Kimura, M. (ed.), *Molecular Evolution, Protein Polymorphism and the Neutral Theory*, Japan Scientific Society Press, Tokyo, and Springer-Verlag, Berlin.

Harris, H., and Hopkinson, D. A. (1978). *Handbook of Enzyme Electrophoresis in Human Genetics*, North-Holland, Amsterdam.
Harris, H., Hopkinson, D. A., and Edwards, Y. H. (1977). Polymorphism and the subunit structure of enzymes: A contribution to the neutralist-selectionist controversy. *Proc. Natl. Acad. Sci. USA* **74**:698.
Hochachka, P. W., and Somero, G. N. (1984). *Biochemical Adaptation*, Princeton University Press, Princeton, N.J.
Hopkinson, D. A., Edwards, Y. H., and Harris, H. (1976). The distribution of subunit numbers and subunit sizes of enzymes: A study of the products of 100 human loci. *Ann. Hum. Genet.* **39**:383.
Johnson, G. B. (1971). Metabolic implications of polymorphism as an adaptive strategy. *Nature* **232**:347.
Johnson, G. B. (1973). Importance of substrate variability to enzyme polymorphism. *Nature New Biol.* **243**:151.
Johnson, G. B. (1974). Enzyme polymorphism and metabolism. *Science* **184**:28.
Johnson, G. B. (1976). Genetic polymorphism and enzyme function. In Ayala, F. J. (ed.), *Molecular Evolution*, Sinauer Associates, Sunderland, Mass.
Kimura, M. (1968). Evolutionary rate at the molecular level. *Nature* **217**:624.
Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*, Cambridge University Press, London.
Kimura, M., and Ohta, T. (1974). On some principles governing molecular evolution. *Proc. Natl. Acad. Sci. USA* **71**:2848.
Koehn, R. K., and Eanes, W. F. (1977). Subunit size and genetic variation of enzymes in natural populations of *Drosophila*. *Theor. Pop. Biol.* **11**:330.
Koehn, R. K., and Eanes, W. F. (1978). Molecular structure and protein within and among populations. *Evol. Biol.* **11**:39.
Kojima, K., Gillespie, J., and Tobari, Y. N. (1970). A profile of *Drosophila* species enzymes assayed by electrophoresis. I. Numbers of alleles, heterozygosities and linkage disequilibrium in glucose metabolizing systems and some other enzymes. *Biochem. Genet.* **4**:627.
Lehninger, A. L. (1975). *Biochemistry*, Worth, New York.
Nei, M., Fuerst, P. A., and Chakraborty, R. (1978). Subunit molecular weight and genetic variability of proteins in natural populations. *Proc. Natl. Acad. Sci. USA* **75**:3359.
Nelson, K., and Hedgecock, D. (1980). Enzyme polymorphism and adaptive strategy in the decapod Crustacea. *Am. Nat.* **116**:238.
Nevo, E. (1978). Genetic variation in natural populations: patterns and theory. *Theor. Pop. Biol.* **13**:131.
Nevo, E. (1983). Adaptive significance of protein variation. In Oxford, G. S., and Rollinson, D. (eds.), *Protein Polymorphism: Adaptive and Taxonomic Significance*, Academic Press, London.
Powell, J. R. (1975). Protein variation in natural populations of animals. *Evol. Biol.* **8**:79.
Quicke, D. L. J., and Brace R. C. (1983). Phenotypic and genotypic spacing within an aggregation of the sea anemone, *Actinia equina*. *J. Mar. Biol. Assoc. U.K.* **63**:493.
Ruth, R. C., and Weid, F. (1976). The subunit structure of glycolytic enzymes. *Comp. Biochem. Physiol.* **54B**:1.
Selander, R. K. (1976). Genetic variation in natural populations. In Ayala, F. J. (ed.), *Molecular Evolution*, Sinauer Associates, Sunderland, Mass.
Smith, P. J., and Fujio, Y. (1982). Genetic variation in marine teleosts: High variability in habitat specialists and low variability in habitat generalists. *Mar. Biol.* **69**:7.
Ward, R. D. (1977). Relationship between enzyme heterozygosity and quaternary structure. *Biochem. Genet.* **15**:123.
Ward, R. D. (1978). Subunit size of enzymes and genetic heterozygosity in vertebrates. *Biochem. Genet.* **16**:779.
Ward, R. D., and Skibinski, D. O. F. (1988). Evidence that mitochondrial enzymes are genetically less variable than cytoplasmic enzymes. *Genet. Res. Cambr.* **51**:121.
Zouros, E. (1975). Electrophoretic variation in allozymes related to function or structure? *Nature* **254**:446.
Zouros, E. (1976). Hybrid molecules and the superiority of the heterozygote. *Nature* **262**:227.