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DIVERGENCE IN ALLOPATRY: MOLECULAR AND MORPHOLOGICAL DIFFERENTIATION BETWEEN SEA URCHINS SEPARATED BY THE ISTHMUS OF PANAMA

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How rapidly do populations separated by a geographic barrier diverge? The question is important to evolutionary biology, because differentiation between such isolates is held to result in the formation of new species (Mayr, 1963; Dobzhansky, 1970; Bush, 1975; Lewontin, 1975). However, there is a lack of direct evidence relevant to the problem because of the difficulties involved in assessing the efficacy of a suspected barrier to genetic exchange. The obstacle to migration, in addition to being nearly absolute, must have been erected recently enough to allow the study of changes at the level of populations rather than major taxonomic groups. It must have also remained in place to the present day so that the populations have not had a chance to either fuse (if reproductive isolation has not developed) or evolve in response to each other (if they have become different species).

A geological event that fulfills these conditions is the Pleistocene emergence of a land bridge between North and South America, which fractionated the range of neotropical marine species sometime between 2 and 5.7 million years ago (Woodring, 1966; Emiliani et al., 1972; Saito, 1976; Webb, 1978). The closure of the portals connecting the tropical Atlantic and Pacific oceans has set the stage for an "evolutionary experiment"; it provides the opportunity to assess the consequences of independent evolutionary development of populations that have remained isolated for a defined period of time. Many genera, belonging to various marine taxa, are represented on both sides of Central America. The Atlantic and Pacific populations of each genus are either placed in the same species, or (quite often) in two separate but closely related species, known as geminates (Jordan, 1908; Ekman, 1953; Rosenblatt, 1963; Rubinoff, 1968). Among these, the shallow-water regular echinoids are particularly interesting in that all seven genera found in the Caribbean are also represented in the eastern Pacific. Taxonomists have recognized the morphological resemblance of Atlantic and eastern Pacific species and have assumed that they comprise geminate pairs (Mortensen, 1928–1951; Mayr, 1954; Chesher, 1972). That Atlantic and Pacific representatives of each genus are assigned different specific names need not imply that reproductive isolation between them has been attained. Though these populations might fuse if the geographic barrier were to be removed (possibly through the construction of a sea-level canal), they have remained spatially separated for at least two million years; how much they have diverged is a question of interest to evolutionary biology.

Of the seven amphi-isthmian genera of sea urchins, I studied three: *Eucidaris, Diadema* and *Echinometra*. *Eucidaris* is represented in the eastern Pacific by *E. thouarsi* (Valenciennes), distributed along the American west coast from lower California to the Galapagos. Its Atlantic geminate, *E. tribuloides* (Lamarck), occurs from South Carolina and Bermuda south to Brazil and east to the African coast. *Diadema* is represented in the eastern Pacific by *D. mexicanum* A. Agassiz, ranging from the Gulf of California to the Ga-

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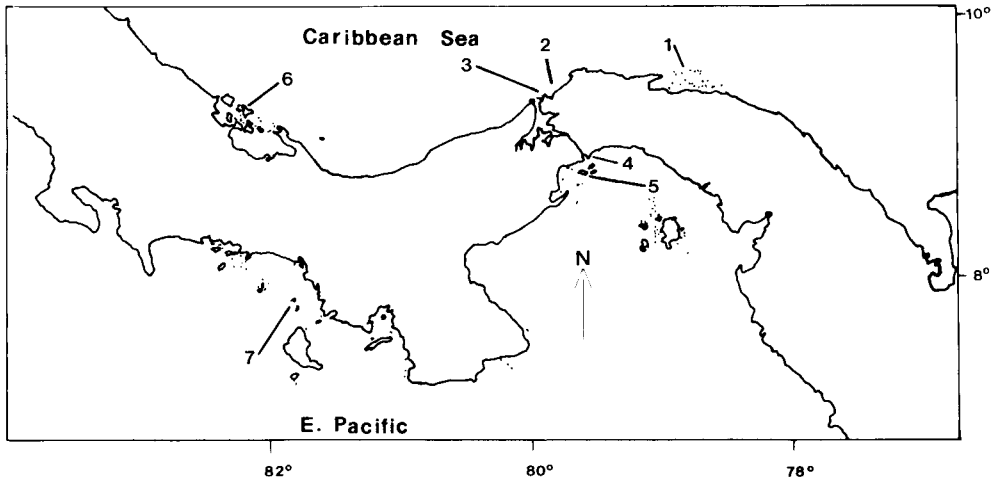

FIG. 1. Localities where sea urchins were collected for the electrophoretic and the morphometric study. *East:* (1) San Blas Islands (*Eucidaris tribuloides* and *Echinometra viridis*), (2) Maria Chiquita (*Echinometra lucunter*), (3) Fort Randolph (*Diadema antillarum*), (4) Punta Paitilla (electrophoretic sample of *Eucidaris thouarsi*), (5) Isla Uraba and Isla Taboguilla (*Diadema mexicanum* and morphometric sample of *E. thouarsi*); *West:* (6) Bocas del Toro (all Atlantic species), (7) Isla Uva (all Pacific species).

lapagos. The Atlantic *D. antillarum* Philippi is found from Florida and Bermuda to Surinam and east to the Gulf of Guinea. *Echinometra* has one species, *E. vanbrunti* A. Agassiz, on the west coast of America, distributed from central California to Peru, and two species, *E. lucunter* (Linnaeus) (Florida and Bermuda to Brazil, east to Dakar and Angola) and *E. viridis* A. Agassiz (restricted to the Caribbean) on the east coast. *Echinometra lucunter* is presumed to be the geminate of *E. vanbrunti* (Mayr, 1954; Chesher, 1972), but the two Atlantic species resemble each other so closely (Mortensen, 1928–1951) that the matter must be regarded as an open question. I have, therefore, included both *E. lucunter* and *E. viridis* in this study.

Protein differences between Atlantic and Pacific members of each echinoid geminate pair have been used to test the hypothesis that proteins evolve at constant rates (Lessios, 1979*a*). The same electrophoretic data are used here in conjunction with morphometric evidence to estimate the degree to which isolated populations tend to diverge on different levels of integration. Though differences in gene products permit inferences about the differentiation of structural genes, they can provide no information on the epigenetic, epistatic and pleiotropic interactions that result in the phenotype. The recent evidence suggesting that regulatory loci may be important in evolution (Maxson and Wilson, 1974, 1975; Wilson et al., 1974*a*, 1974*b*; King and Wilson, 1975; Wilson, 1976) emphasizes the need to approach questions of evolutionary divergence on both molecular and morphological grounds. Morphological evidence may give a better overview of the similarities and differences between the isolates, but can provide no direct information about the genetic underpinnings of this variability. The two approaches are combined here to address the following questions: (1) How much have the geminate species diverged on the molecular and the morphological level? (2) Is there any congruence between the extent of differentiation on the two levels? (3) What factors can be implicated as potentially important agents of divergence on each level?

MATERIALS AND METHODS

Two populations were studied for each species; the specimens used for the electrophoretic sample came from the same

TABLE 1. *Tissue used and number of loci scored in the electrophoretic study.*

Enzyme	Tissue	Number of loci scored		
		Eucidaris	*Diadema*	*Echinometra*
Am	Gut	2	2	3
Est	Gut	3	2	—
G6PDH	Gut	—	1	1
HK	Muscle	1	1	1
LAP	Gut	1	1	2
MDH	Muscle	1	2	2
M6PI	Muscle	1	1	1
Pep	Gut	1	2	2
PGI	Muscle	1	1	1
PGM	Muscle	1	2	2
TO	Gut	1	1	1
TPI	Muscle	1	1	1
XDH	Gut	1	1	1
Total no. of loci		15	18	18

locality (or from an immediately adjacent one) as the ones used in the morphometric study (Fig. 1). I refer to the samples from the vicinity of the Panama Canal as the "eastern" ones and to those from the Gulf of Chiriqui (Pacific) and Bocas del Toro (Atlantic) as the "western" ones.

Electrophoresis was performed in 18 × 14 × 1 cm slabs of 11% polymerized starch (Otto Hiller, Lot 307) for all assays except amylase; 7% polyacrylamide gels (8 × 6 × 0.03 cm) were used for the latter. Sample size per locus ranged from 18 to 66 individuals, with one exception, phosphoglucomutase in the western sample of *Eucidaris thouarsi,* consisting of only three; most loci were assayed in more than 45 sea urchins per population. The samples consisted of either gut or muscle from the jaw system. They were assayed for the following enzymes: Amylase (Am), esterases (EST), glucose-6-phosphate dehydrogenase (G-6-PDH), hexokinase (HK), leucine amino peptidase (LAP), NAD-dependent malate dehydrogenase (MDH), mannose-6-phosphate isomerase (M6PI), peptidases (Pep), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), tetrazolium oxidase (TO), triosephosphate isomerase (TPI) and xanthine dehydrogenase (XDH). The kind of tissue used and the number of presumptive loci scored in each enzyme assay are presented in Table 1. Running and staining buffers are mostly those of Ayala et al. (1972, 1974*b*) and Marcus (1977). Detailed descriptions of the methods are given in Lessios (1979*b*). Molecular divergence was quantified with Nei's (1975) standard genetic distance.

TABLE 2. *Characters used in morphometric study. See Durham and Wagner (1966) for definition of anatomical terms and Lessios (1979b) for methods of measurement.*

1. Longest diameter of the test at the ambitus (LAXIS)
2. Diameter of the test perpendicular to LAXIS (SAXIS)
3. Height of the test (HEIGHT)
4. Diameter of the apical system (APDIAM)
5. Width of genital plate 5 (GENWID)
6. Length of genital plate 5 (GENLEN)
7. Width of ocular plate III (OCWID)
8. Length of ocular plate III (OCLEN)
9. Number of tubercles on genital plate 5 (GENSP)
10. Diameter of the peristome (PERSTM)
11. Maximum width of the ambulacrum (AMBW)
12. Maximum width of the interambulacrum (INTW)
13. Pore pairs per plate at the ambitus (PPP)
14. Number of ambulacral plates in a series (AMBP)
15. Number of interambulacral plates in the series adjacent to the ambulacrum (INTP)
16. Tooth length (TOOTHL)
17. Maximum tooth width (TOOTHW)
18. Height of the symphysis between auricles of the perignathic girdle (SYMPH)[1]
19. Diameter of ambulacral areole at the ambitus (AMBAR)[1]
20. Horizontal diameter of interambulacral areole at the ambitus (INTARH)
21. Vertical diameter of interambulacral areole at the ambitus (INTARV)
22. Diameter of ambulacral primary mamelon at the ambitus (AMBMA)
23. Diameter of interambulacral primary mamelon (INTMA)

[1] Absent in *Eucidaris*.

The morphometric study is based on 23 characters (21 for *Eucidaris*) examined in 40 individuals from each species (20 from each population). A brief description of each is given in Table 2. These characters pertain to all the parts of sea urchin skeletal anatomy except the pedicellariae. I treated the morphometric data in two ways to answer two related, but not identical, questions. To quantify morphological dissimilarity between populations I calculated the Mahalanobis (1936) generalized distance coefficient D^2. To find out

TABLE 3. *Nei's standard genetic distance* D *(below the diagonal) and Mahalanobis generalized distance* $\sqrt{D^2}$ *(above the diagonal) between populations of* Eucidaris. *Values of Nei's index in parentheses are those obtained from an analysis restricted to the twelve loci common to all genera studied. Probability levels under the Mahalanobis distances refer to the multivariate* F *statistic for equality of means.*

		Atlantic		Pacific	
		E. tribuloides		*E. thouarsi*	
		East	West	East	West
E. tribuloides	East	—	4.309 **	4.071 **	4.279 **
	West	.016 (.021)	—	4.752 ***	6.789 ***
E. thouarsi	East	.292 (.400)	.307 (.419)	—	5.164 ***
	West	.357 (.480)	.360 (.482)	.024 (.028)	—

Mean Nei's distance:		Mean Mahalanobis distance:	
within species:	.020 (.025)	within species:	4.737
between species:	.329 (.445)	between species:	4.972

** $P < .01$, *** $P < .001$.

how well Atlantic and Pacific species of each genus can be distinguished from each other, I used discriminant analysis.

The square root of the Mahalanobis distance is a measure of the distance between the centroids of two groups in multivariate space with the axes tilted with respect to each other to account for correlations between characters and stretched in inverse proportion to the variance of each character. Though the measure itself may be rather robust to deviations from multivariate normality, the mathematical justification of the Mahalanobis distance assumes that the data conform to the multivariate normal distribution (Sneath and Sokal, 1973). Discriminant analysis is the procedure of creating a linear classification function by weighing characters so that their combination has maximal variance between groups relative to the pooled variance within groups. The weights are calculated so as to compensate for redundancy of information due to intercorrelations between measurements. In addition to the assumption that the clusters have multivariate normal distributions, the use of discriminant analysis also rests on the premise that their dispersion matrices are homogeneous (Sneath and Sokal, 1973).

Of the characters examined, pore pairs per plate varies between individuals of the same species in *Echinometra,* but it is constant in the order Cidaroida, as it is in the genus *Diadema.* In order to avoid biasing the estimates of relative divergence in favor of *Echinometra* by including a character known a priori not to vary in the other two, PPP was not used in the calculation of Mahalanobis distances for any genus. Similarly, the two characters missing in *Eucidaris* (AMBAR and SYMPH) were eliminated from the calculation of the distances in the other two genera, in the interest of preserving the comparability of the indices. Mahalanobis distances are, therefore, calculated on the basis of 20 characters, all of which are homologous in the three genera. Discriminant analysis is meant to answer the question of whether the geminates can be distinguished from each other; conspecific populations were therefore pooled and all available characters were used.

Computer program BMDP3D (Fu and Douglas, 1977) was used to calculate the Mahalanobis distances between the pop-

TABLE 4. *Nei's standard genetic distance* D *(below the diagonal) and Mahalanobis generalized distance* $\sqrt{D^2}$ *(above the diagonal) between populations of* Diadema. *Values of Nei's index in parentheses are those obtained from an analysis restricted to the twelve loci common to all three genera studied. Probability levels under the Mahalanobis distances refer to the multivariate* F *statistic for equality of means.*

		Atlantic		Pacific	
		D. antillarum		*D. mexicanum*	
		East	West	East	West
D. antillarum	East	—	8.761 ***	8.944 ***	11.204 ***
	West	.036 (.038)	—	6.161 ***	5.000 ***
D. mexicanum	East	.040 (.052)	.016 (.023)	—	7.321 ***
	West	.039 (.046)	.008 (.012)	.015 (.024)	—

Mean Nei's distance:		Mean Mahalanobis distance:	
within species:	.026 (.031)	within species:	8.041
between species:	.026 (.033)	between species:	7.827

** $P < .01$, *** $P < .001$.

ulations; program BMDP7M (Jennrich and Sampson, 1977) was employed to carry out the discriminant analysis. The latter program also calculates the posterior probability that each specimen belongs to a group on the basis of a discriminant function determined from all other individuals; it generates "jackknifed" identification matrices, thus providing a measure of the success of the classification and the degree to which the groups can be distinguished from each other.

RESULTS

Electrophoretic differentation.—The allele frequencies in every presumptive locus are given in Lessios (1979*b*). Nei's standard genetic distances between the populations of each genus are presented in Tables 3, 4, and 5. Values calculated from the 12 loci that are common in all the genera are not substantially different from those produced by an analysis based on all the available data for each genus (Tables 3–5). Nei's index values, however, are only meaningful in a relative sense. The best method of measuring transisthmian divergence in each genus is to compare it to differentiation between its populations on the same coast. This standard solves, to a certain extent, the problem that would arise if "hidden variation" (Singh et al., 1975, 1976; Coyne, 1976; Milkman, 1976; Coyne et al., 1978, 1979) were unequally distributed among the genera. Its major problem is that it assumes that intra- and interspecific differences would increase linearly with respect to each other if populations farther apart within the range of each species were sampled. Such an assumption, tentative as it may be, is still safer than the one—implicit in any other standard—that the few samples taken give reliable estimates of gene frequencies for the entire species. Measured with the yardstick of differentiation between local populations, the three echinoid species pairs show striking differences in divergence. Pacific populations of *Diadema* have not diverged from their Atlantic counterparts any more than they have from populations on the same coast (Table 4). *Eucidaris* and *Echinometra*, on the other hand, exhibit transisthmian distances 16 and 37 times larger than intraspecific ones (Tables 3 and 5). Even if we consider intraspecific values of Nei's index as roughly equal, we have to accept that interoceanic divergence in *Echinometra* is on the average 20 times

TABLE 5. *Nei's standard genetic distance* D *(below the diagonal) and Mahalanobis generalized distance* $\sqrt{D^2}$ *(above the diagonal) between populations of* Echinometra. *Values of Nei's index in parentheses are those obtained from an analysis restricted to the twelve loci common to all three genera studied. Probability levels under the Mahalanobis distances refer to the multivariate* F *statistic for equality of means.*

		Atlantic				Pacific	
		E. lucunter		*E. viridis*		*E. vanbrunti*	
		East	West	East	West	East	West
E. lucunter	East	—	5.340 ***	10.931 ***	13.220 ***	8.161 ***	6.782 ***
	West	.009 (.012)	—	10.038 ***	12.794 ***	6.283 ***	4.423 ***
E. viridis	East	.117 (.180)	.109 (.169)	—	4.266 **	12.247 ***	10.901 ***
	West	.117 (.177)	.111 (.172)	.007 (.008)	—	14.874 ***	13.631 ***
E. vanbrunti	East	.556 (.655)	.531 (.649)	.620 (.771)	.612 (.790)	—	5.310 ***
	West	.561 (.658)	.547 (.672)	.666 (.847)	.653 (.854)	.021 (.032)	—

Mean Nei's distance:		Mean Mahalanobis distance:	
E. lucunter-E. vanbrunti		*E. lucunter-E. vanbrunti*	
within species:	.015 (.022)	within species:	5.325
between species:	.549 (.659)	between species:	6.412
E. lucunter-E. viridis		*E. lucunter-E. viridis*	
within species:	.008 (.010)	within species:	4.803
between species:	.114 (.175)	between species:	11.746
E. vanbrunti-E. viridis		*E. vanbrunti-E. viridis*	
within species:	.014 (.020)	within species:	4.788
between species:	.638 (.816)	between species:	12.913

** $P < .01$, *** $P < .001$.

larger than in *Diadema*. This conclusion holds whether *E. vanbrunti* is compared to either *E. lucunter* or *E. viridis*. It is unlikely that these differences in divergence are the artifacts of the limited resolving power of electrophoresis; if the only cause of these differences were that the employed buffers detected more variability in *Echinometra* and *Eucidaris* than in *Diadema,* the former two genera should also exhibit higher apparent values of intraspecific differentiation and heterozygosity; such patterns are not evident (Lessios, 1979*a*).

Another means of judging the magnitude of divergence between the populations under comparison is to relate it to the differentiation from a third species in the same group (Hubby and Throckmorton, 1968). *Echinometra viridis* and *E. lucunter* are closely related but distinct species. That they are good species is evident from their morphological differences (Mortensen, 1928–1951; McPherson, 1969; this article), their ecological separation (Mayr, 1954; Kier and Grant, 1965), and by the finding of the present survey that in one locus, *Am-1,* they do not share any alleles (Lessios, 1979*b*). The genetic distance between *Echinometra lucunter* and *E. viridis* is on the average five times smaller than the distance between either of them and *E. vanbrunti*. The transisthmian distance in *Eucidaris* is about three times larger than the distance between the sympatic species of *Echinometra,* and in *Diadema* it is roughly four times smaller.

A third standard of comparison, less re-

TABLE 6. *Percentage of loci in each interval of Nei's genetic identity* I. *Gene frequencies of conspecific populations have been pooled.*

	Genetic identity									
	0–.09	.10–.19	.20–.29	.30–.39	.40–.49	.50–.59	.60–.69	.70–.79	.80–.89	.90–1
Diadema	0	0	0	0	0	0	0	0	11	89
Eucidaris	13	0	7	0	7	7	0	0	0	67
Echinometra										
E. vanbrunti-E. lucunter	33	0	0	0	0	6	0	0	0	61
E. vanbrunti-E. viridis	17	17	0	0	0	11	6	11	6	33
E. lucunter-E. viridis	6	0	0	0	0	0	0	11	17	67

liable but widely used (e.g., Johnson and Selander, 1971; Johnson et al., 1972; Turner, 1974; King and Wilson, 1975; Greenbaum and Baker, 1976; Nixon and Taylor, 1977) is to compare divergence in the populations studied to that in other groups for which data exist. If we use as our standard the extensive study of Ayala et al. (1974*a*), we find that the differentiation of the species of *Echinometra* from the two coasts of Central America is roughly equivalent to that of sibling species in the *Drosophila willistoni* group, the divergence between the geminates of *Eucidaris* is slightly larger than that of subspecies or semispecies, and that the two *Diadema* "species" have diverged no more than local populations of *Drosophila*. If divergence in other echinoderms is used as a yardstick, the conclusions are the same: *Echinometra vanbrunti* has diverged from *E. lucunter* about as much as *Asterias vulgaris* has from its closely related but morphologically distinct congener *A. forbesi* (Schopf and Murphy, 1973), while *Diadema mexicanum* and *D. antillarum* are more similar to each other than local populations of *Arbacia punctulata* (Marcus, 1977).

Thus, whatever standard of comparison is used, the results are the same: geminates of *Echinometra* show considerable values of genetic divergence, those of *Eucidaris* are intermediate, while Atlantic and Pacific *Diadema* populations are remarkable for their similarity.

If the distribution of the loci with respect to genetic identity is tabulated (Table 6), in *Echinometra* it exhibits the U-shaped pattern characteristic of comparisons between good species, while in *Diadema* it takes the form displayed by conspecific populations of most animals (Ayala, 1975; Avise, 1976). The differences between the number of loci in each identity class in *Diadema, Eucidaris* and transisthmian comparisons of *Echinometra* are highly significant (Kruskal-Wallis, $H_c = 363.35$, $P < .001$).

Morphometric differentiation.—Matrices of intra- and interspecific Mahalanobis distances (Tables 3–5) indicate that geographical variation in morphology within each species is almost comparable to variation between species. Though differences of multivariate means are highly significant between species, the same holds true for comparisons within species. In every genus there is at least one population which is less similar to its conspecific population than it is to one on the opposite coast of the Isthmus. The opinion of classical taxonomists that, on morphological grounds, Atlantic and Pacific species should be considered members of geminate pairs (Mayr, 1954; Chesher, 1972) is, therefore, confirmed by quantitative data. Similarly, the subjective impression that *Echinometra lucunter* is the most closely related species to *E. vanbrunti* (Mortensen, 1928–1951) is supported.

If interspecific distances in each genus are compared to intraspecific ones, populations of *Diadema* on opposite coasts of the Isthmus seem less different than populations on the same coast (Table 4), populations of *Eucidaris* in different oceans appear as similar to each other as they are to other populations in the same ocean (Table 3), while populations of *Echino-*

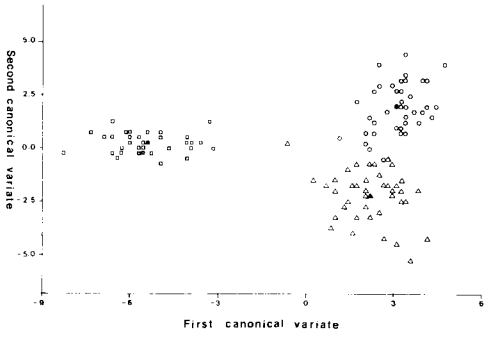

FIG. 2. Plot of the two canonical variates for the three species of *Echinometra*. Circles: *E. lucunter*; triangles: *E. vanbrunti*; squares: *E. viridis*. Filled symbols indicate the mean of each group.

metra vanbrunti and *E. lucunter* seem, on the average, to be slightly more distinct between species than they are within species (Table 5). It would be interesting to know whether these differences are statistically significant, but, although D^2 can be used for a multivariate test of equality of means, no test has been devised to compare one D with another, let alone a "relative average D" with the next (Atchley, 1978).

If we adopt this relative standard of divergence and assume that these differences between the genera are not due to chance, we can conclude that transisthmian differentiation on the morphological level follows the same trend as molecular divergence. In contrast to the differences in molecular divergence, however, discrepancies between the genera in degree of morphological differentiation are slight. Furthermore, the morphometric differences between the two sympatric species of *Echinometra* are entirely discordant with the pattern displayed by electrophoretic dissimilarities. While on the molecular level *E. viridis* has diverged little from *E. lucunter,* the morphological distances between their populations are, on the average, twice as large as the intraspecific distances, a magnitude of differentiation which surpasses that of *E. lucunter* to *E. vanbrunti*. The marked dissimilarity of *E. viridis* to either congener is more distinctly illustrated in a plot of the three species (conspecific populations pooled) on the two canonical variates generated by the discriminant analysis (Fig. 2).

Jackknifed identification matrices based on the classification functions of the discriminant analysis indicate that it can distinguish between the species of each genus with a good deal of success (Table 7). Ninety-two percent of *Echinometra lucunter* and *E. vanbrunti* can be classified correctly by discriminant functions calculated on the basis of all individuals except the one being classified, while *E. viridis* is so distinct that none of its individuals is misclassified. The percentage of correct identifications is somewhat lower in the other two genera, but still satisfactory. Thus, even though the geminate species have not diverged extensively on the morphological level, they do not resemble each other so much that they cannot be told apart if a combination of enough characters is employed.

To determine the degree to which the choice of characters and the relative weight of each in the discriminant function depends on the particular populations used to create them, I calculated classification functions using one of the populations in each species, and I determined the percentage of specimens from the other population which can be classified correctly on their basis (Table 8). Ninety-five percent of all specimens of *Echinometra viridis* can be assigned to the correct species in this manner, but the success of classification across the Isthmus is poor: 63% of the specimens of *Echinometra lucunter* and *E. vanbrunti,* 66% of *Eucidaris,* and 53% of *Diadema* can be put in the right group. Thus, the variation that helps distinguish the geminates from each other is, as the Mahalanobis distances also suggest, of roughly the same magnitude as the variation between populations of the same species.

DISCUSSION

While degrees of molecular divergence between Atlantic and Pacific species in each genus are remarkable for their dif-

TABLE 7. *Jackknifed identification matrices. Each specimen is classified by a discriminant function determined on the basis of all other individuals in the genus.*

Real species affiliation	Number of specimens classified into species			
	Echinometra			
	E. lucunter	*E. viridis*	*E. vanbrunti*	% correct
E. lucunter	37	1	2	92.5%
E. viridis	0	40	0	100.0%
E. vanbrunti	3	0	37	92.5%
			Total	95.0%
	Eucidaris			
	E. tribuloides	*E. thouarsi*		
E. tribuloides	35	5		87.5%
E. thouarsi	7	33		82.5%
			Total	85.0%
	Diadema			
	D. antillarum	*D. mexicanum*		
D. antillarum	33	7		82.5%
D. mexicanum	2	38		95.0%
			Total	88.7%

ferences, morphological differentiation between the same species has been slight. That this resemblance is not an artifact of the accidential choice of invariant characters is indicated by the larger distances between *Echinometra viridis* and its conspecifics and by the discriminant analysis, which reveals that Atlantic populations of each genus can be distinguished successfully from Pacific ones. Discriminant analysis, however, also shows that the variation which aids in separating the geminates from each other is not substantially different from local variation within each species.

Had these species not belonged to the biogeographic entity of the geminate pairs of Panama, we could have easily concluded that, according to the hypothesis of the molecular clock (Wilson et al., 1977), dissimilarities in divergence of structural genes are indicative of different times of separation, and that the morphological resemblance within *Echinometra* and *Eucidaris* is the result of convergence or parallelism on the organismal level. However, even the strongest supporters of the molecular clock admit exceptions to the proposed rule of a linear relationship between time since separation and extent of molecular differentiation (Wilson et al., 1977). Reasons for doubting the validity of the dates of separation estimated on the assumption that proteins evolve at constant rates have been presented elsewhere (Lessios, 1979*a*). The low values of morphological divergence indicate that, as Mayr (1954) and Chesher (1972) have assumed, the rise of the Isthmus is a probable cause of separation between Atlantic and Pacific species of each genus. If, as the molecular clock would require, the Atlantic and Pacific species of only one of the three genera were separated by the establishment of the Isthmus, it is hard to explain how speciation events unrelated to the closure of the portals connecting the two oceans left the surviving species with virtually identical ranges and with a morphologically closely related form of each on the opposite side of Central America. To be sure, an elaborate scenario could be constructed according to which such speciation events due to unknown geological occurrences were coupled with range invasions, selective extinctions and subsequent anatomical convergence of the surviving forms to result in the present-day distribution and

TABLE 8. *Success of identification when discriminant function in each genus is determined on the basis of populations other than the ones being classified. A, C, E: Samples of Western populations classified by discriminant functions determined on the basis of eastern populations of the same genus. B, D, F: Samples of Eastern populations classified by discriminant functions based on Western ones.*

Real species affiliation	Number of specimens classified into species			
	Echinometra (A)			
	E. lucunter	*E. viridis*	*E. vanbrunti*	% correct
E. lucunter	8	3	9	40%
E. viridis	1	18	1	90%
E. vanbrunti	4	0	16	80%
			Total	70%
	Echinometra (B)			
	E. lucunter	*E. viridis*	*E. vanbrunti*	
E. lucunter	14	2	4	70%
E. viridis	0	20	0	100%
E. vanbrunti	7	0	13	66%
			Total	78%
	Eucidaris (C)			
	E. tribuloides	*E. thouarsi*		
E. tribuloides	13	7		65%
E. thouarsi	4	16		80%
			Total	72.5%
	Eucidaris (D)			
	E. tribuloides	*E. thouarsi*		
E. tribuloides	17	3		85%
E. thouarsi	13	7		35%
			Total	60%
	Diadema (E)			
	D. antillarum	*D. mexicanum*		
D. antillarum	18	2		90%
D. mexicanum	20	0		0%
			Total	45%
	Diadema (F)			
	D. antillarum	*D. mexicanum*		
D. antillarum	5	15		25%
D. mexicanum	1	19		95%
			Total	60%

morphological resemblance of the species. However, *Eucidaris, Echinometra* and *Diadema* are not isolated cases of morphologically similar forms, one on each side of Central America, but part of the vast array of genera, belonging to many taxa, that contain geminate species (Jordan, 1908; Ekman, 1953; Rosenblatt, 1963; Rubinoff, 1968). It is hard to believe that two out of the three genera are exceptions to the general biogeographic pattern, and yet that their populations from each coast came to resemble each other so closely. The most parsimonious explanation for this pattern is that separation between the members of each pair resulted from the Isthmus emergence and that rates of molecular evolution have not been uniform in Panamanian echinoids.

Cases of congruence between morpho-

logical and molecular divergence between taxa (Hubby and Throckmorton, 1968; Patton et al., 1975) could perhaps be best explained as the products of correlation between degree of differentiation on each level and the time that the lineages have remained separate (Wilson, 1976). In the geminate species with which we are dealing, however, the congruence between the two sets of characters is poor. Even if the assumption of simultaneous separation between species is wrong, we still have to ask what factors would account for the observed discrepancy in degrees of differentiation on each level of integration.

The incongruence between extent of divergence in the two sets of characters could be the result of different sensitivities of each to different components of the environment. Isozyme patterns are in all likelihood genetically determined and, if not selectively neutral, they are more likely to be influenced by physical variables such as temperature (Koehn and Ramussen, 1967; Johnson, 1971; Schopf and Gooch, 1971; Koehn et al., 1976) and salinity (Koehn et al., 1976); morphological variation, on the other hand, involves a large developmental component in addition to its genetic underpinnings and, in sea urchins, it is likely to reflect the type of substratum each species occupies (Mortensen, 1928–1951; Oldfield, 1976). Generation time, mutation pressure and population size have been examined by Lessios (1979*a*) as possible explanations of the observed degrees of molecular divergence; they were rejected in favor of the alternative hypothesis that natural selection can best account for the different degrees of divergence but similar levels of heterozygosity found in the three echinoid genera. To find out exactly how selection brought about the present-day molecular and morphological divergence patterns one would need a complete reconstruction of the environment from the Pleistocene to the Recent in addition to the knowledge of the genetic structure of the populations and the ways in which the two interact. Such information is not available, but what is known about the biology of these animals may be used to point out some suggestive correlations between degree of molecular and morphological divergence and ecological habits of each genus.

The three genera studied here differ in bathymetric range and degree of ecological specialization. *Diadema* is ecologically an extreme generalist. It is ubiquitous in both shallow and deeper water (Kier and Grant, 1965) and reaches a depth of 400 m (Mortensen, 1928–1951). It also occupies a variety of habitats, displaying high abundances on rock, live and dead coral, mangrove roots, seagrass beds and sand (Randall et al., 1964; Kier and Grant, 1965). If a correlation exists between morphology and type of substratum, it is not surprising that *Diadema* is also morphologically more plastic and exhibits higher local variation than the other genera both within and between species. Interspecific distances are smaller than intraspecific ones, possibly because there is a higher degree of substratum equivalence between localities in the two oceans than there is between localities on the same coast. Thus, the smallest Mahalanobis distance in *Diadema* is the one between western populations of *D. antillarum* and *D. mexicanum,* the two sites where live coral reef predominates; the population most different from all others is the one from the eastern locality of *D. antillarum,* where it was taken at the sandy lagoon bottom (Table 4). *Diadema,* by virtue of its wide bathymetric distribution, could also be less affected by the pronounced physical differences of the shallow-water environments of the two oceans (Glynn, 1972), differences that do not extend to deeper water. Being a generalist, *Diadema* may also perceive the physical environment as more fine-grained, so that it would not need to adapt genetically to what may constitute major differences for the other two genera. Selective pressures, therefore, on the structural genes of *Diadema* may have been similar in both the Atlantic and the eastern Pacific, which might account for the small degree of molecular divergence of populations from the two oceans.

The species of *Eucidaris* and *Echino-*

metra have more restricted bathymetric ranges and more specialized ecological requirements than those of *Diadema*. *Echinometra lucunter* and *E. vanbrunti* are almost entirely limited to the intertidal (Mortensen, 1928–1951; Kier and Grant, 1965; McPherson, 1969); *Eucidaris* reaches its highest abundance in the intertidal zone (Mortensen, 1928–1951; Clark, 1933; McPherson, 1968) but occasionally ranges down to 450 m in the Atlantic and 45 m in the eastern Pacific (Mortensen, 1928–1951). Both *Echinometra* and *Eucidaris* are limited to hard substrata, regardless of locality. Their intraspecific morphological distances are, therefore, smaller than those of *Diadema,* and, because the nature of hard substrata other than coral in the two oceans differ (volcanic rock in the eastern Pacific *versus* limestone in the Caribbean), the interspecific morphological distances are by comparison larger, the more so between *Echinometra lucunter* and *E. vanbrunti,* which, unlike *Eucidaris,* do not inhabit live coral reef. The two genera are also more exposed to the pronounced physical differences of shallow-water marine environments because of their bathymetrical distributions, the littoral *Echinometra* bearing the full brunt of dissimilar intertidal environments, and consequently exhibiting the highest degrees of molecular divergence. Finally, *Echinometra viridis* and *E. lucunter,* living in the same ocean, are subject to the same overall physical regimes, but they, like other closely related species that are sympatric, occupy different habitats. *Echinometra lucunter* is abundant on intertidal reef flats, while *E. viridis* prefers live coral in the subtidal region down to about 12 m (Kier and Grant, 1965; McPherson, 1969; pers. observ.). That the two species are similar in molecular structure but dissimilar in morphology could, therefore, also be explained as the result of correlation between morphology and substratum on the one hand, and molecular structure and physical variables on the other.

This explanation, based on correlation, is tenuous. More certain is the fact that in these sea urchins, as in many other animals studied (Gould et al., 1974; Johnson, 1974; Maxson and Wilson, 1974; Turner, 1974; Avise et al., 1975; Kornfield and Koehn, 1975; Nolan et al., 1975; Nixon and Taylor, 1977; Schnell et al., 1978; Turner et al., 1979; Larson, 1980; Scanlan et al., 1980) structural genes and external morphology have evolved at different rates. Morphological divergence between allopatric populations has proceeded on the average at a more conservative rate than molecular differentiation, while the converse has been true in the sympatric species of *Echinometra*. If morphological adaptations are dictated by the habitat, while isozymes are either selectively neutral or influenced by physical variables, and if ecological separation is the rule in closely related species invading the same area, then the pattern displayed by these sea urchins of rapid morphological divergence in sympatry relative to molecular differentiation may well be a general phenomenon. Allopatric populations, on the other hand, seem to diverge at varying rates depending on the differences between their respective environments as each species perceives them.

Since this manuscript was submitted, a paper (Vawter et al., 1980) critical of my initial report of the molecular data (Lessios, 1979*a*) has appeared in this journal. Vawter et al. believe that their own data from geminate species of fish provide support for the molecular clock hypothesis, while mine are not necessarily inconsistent with its predictions. I disagree.

Vawter et al.'s study suffers from a serious weakness, not apparent from their report: different proteins were used in the determination of each genetic distance. The molecular clock hypothesis states that *each* protein evolves at a constant rate. It has never been the contention of its supporters that *all* proteins evolve at the same rate. On the contrary, Sarich (1977) has reported that some proteins evolve much faster than others. It follows that to test the clock one must use the same proteins. In a comparison of Nei's indices obtained from the various geminate pairs, loci not

common to all can only be admitted if they do not appreciably alter the average values. Vawter et al. report that they used 22 to 41 loci; they neglect to mention, however, that only six of these are common to all their comparisons. One of these is an esterase, which they state they cannot score reliably, while three more are general proteins, the homologies of which are also problematical. The genetic distances they report, therefore, are only functions of the percent of "fast" and "slow" loci included in the determination of each, and the comparisons between them are meaningless. Moreover, Vawter et al. have proclaimed an arbitrarily restrictive criterion of falsification for the molecular clock hypothesis through the use of the geminate species of Panama, namely, that interoceanic D values must be "as low as or lower than" intraoceanic ones. Though they are correct in saying that high values could result from speciation events predating the rise of the Isthmus and that only low values are acceptable falsifiers, they have not explained why such low values would have to be no larger than the ones between populations which are still exchanging genes. Nor have they considered that values so low would rarely be obtained in populations isolated for a minimum of two million years whether proteins evolve at constant rates or not. That none of the transisthmian genetic distances in Vawter et al.'s comparisons of dissimilar protein samples assayed in 1 to 23 individuals per locality is lower than intraoceanic distances in other species of fish, hardly constitutes strong support for the molecular clock. It should be noted that times of divergence calculated by Vawter et al. from their data do not, as they imply, coincide with the duration of the isthmus emergence, but only with the range of paleontological estimates as to its completion.

It so happens that the average transisthmian divergence of *Diadema* in my study is equal to the average intraspecific genetic distance in this genus, and thus satisfies even Vawter et al.'s excessively restrictive criterion of falsification. Consequently they devoted a major part of their article to criticizing Lessios (1979*a*). The most plausible argument they present is that the 18 loci I assayed do not constitute an adequate sample for testing the clock hypothesis. They calculate from the binomial that if 10% of the entire genome of the two species of *Diadema* were fixed for alternate alleles, there would be a probability of 0.15 that none of these differences would be detected. However, there are endless games one can play in the absence of any theoretical minimum limit on the number of "loci" needed to test the hypothesis that each protein (not each genome) evolves at a constant rate. For example, following Vawter et al.'s reasoning, according to which comparisons of different proteins in different taxa are permissible, "the clock" would expect a Nei's D of 0.214 (the mean of their values) and not 0.11. The expected probability that no difference would be found with 18 loci is not 0.15 but $(0.8)^{18} = 0.018$. There is no doubt that the larger the number of proteins (if they are the same ones), the more general the conclusions; however, no matter what the probability of finding a difference in an unsampled locus might be, the fact remains that for the 12 "loci" common to all the sea urchin species, the mean transisthmian Nei's D value is 0.445 for *Eucidaris* and 0.033 for *Diadema*. Some of these proteins did not diverge at a constant rate and neither did their average.

In Lessios (1979*a*) I presented reasons as to why different degrees of divergence coupled with similar levels of heterozygosity do not meet the predictions of the neutral mutation theory, which ascribes protein differentiation to random processes. I consequently suggested that natural selection is the most plausible explanation for the determined patterns. In an apparent confusion between evolution and divergence, Vawter et al. criticize this suggestion on the grounds that I did not explain "how selection would eliminate all new mutants while preserving polymorphisms." A small degree of differentiation need not mean that all new mutants were eliminated, but simply that the same mu-

tations were removed by selection in the two species. There is no evidence that the polymorphisms of the ancestral stocks were preserved. In contradiction to their dismissal of my selectionistic explanation, Vawter et al. also say that the species of *Diadema* must be unique, because they didn't diverge despite the different climatic and biotic regimes (i.e., selective pressures) they experience. In the present article I suggest one possible manner in which different species living in the same general environment may be subject to different selective pressures. Whether or not this suggestion is correct, the very low divergence in *Diadema* relative to the other genera was not expected by either the molecular clock hypothesis or the neutral mutation theory, and thus encourages a selectionistic explanation.

Though Vawter et al. accuse me of attempting to "bury the clock," my real conclusion was that "the molecular clock hypothesis does not hold for Panamanian echinoids." I believe that this conclusion has not been seriously challenged and should stand, at least until such time as amino acid sequencing shows otherwise. The molecular clock may prove robust over periods of time larger than the 2–5 million year range that the Panamanian geminate species can test. The evidence produced from the geminate species of Panama, however, including data from fish, does not support the premise that the clock keeps good time in the 2–5 million year range.

Summary

The regular echinoid genera *Eucidaris, Diadema* and *Echinometra* are represented on the two sides of Central America by geminate species, believed to have resulted from the emergence of the Isthmus of Panama in the late Pliocene. Divergence between the members of each geminate pair (and of an additional Caribbean species of *Echinometra* from its congeners) was studied electrophoretically and morphometrically in an effort to gain an understanding of the changes in structural genes and external anatomy in populations isolated by a geographic barrier for a known period of time.

Analysis of 18 presumptive loci (15 in *Eucidaris*), encoding a total of 13 enzymatic proteins, revealed pronounced differences in degree of differentiation in the three species pairs. Pacific populations of *Diadema* have diverged from their Atlantic counterparts no more than they have from populations on the same coast. *Eucidaris* and *Echinometra,* on the other hand, exhibit interoceanic genetic distances 16 and 37 times greater than intraspecific ones. Transisthmian distance in *Echinometra* is 20 times larger than it is in *Diadema.* The third species of *Echinometra, E. viridis,* has diverged from its sympatric Caribbean congener, *E. lucunter,* only one-fifth as much as the latter has diverged from its Pacific congener, *E. vanbrunti.*

Morphometric differentiation between the members of each pair, assessed on approximately 20 characters and quantified with the Mahalanobis generalized distance, is not substantially different from local variation within each species. The contention of previous authors that morphological evidence argues for a geminate relationship of these species is, therefore, confirmed. Discriminant analysis indicates that populations of geminate species can be distinguished from each other, but that the variation which aids in this discrimination is not substantially different from local variation within each species.

The ratio (but not the absolute values) of inter- to intraspecific mean Mahalanobis distances is lowest in *Diadema,* intermediate in *Eucidaris,* and highest in *Echinometra,* a pattern that agrees with the one displayed by the average Nei's indices calculated from electrophoretic data. This is the only instance of congruence between molecular and morphological data, and it is limited to interoceanic comparisons. The Caribbean species of *Echinometra* show no concordance between the two sets of characters. While on the molecular level, *E. viridis* has diverged little from *E. lucunter,* the mean morphological distance between them is twice as

large as their mean intraspecific distances, a magnitude of differentiation that surpasses that of *E. lucunter* from the eastern Pacific *E. vanbrunti*. This pattern may result from different sensitivities of each level of integration to different components of the environment: allozyme frequencies may be primarily influenced by physical variables, while morphology is more likely to reflect the type of substratum that each species occupies. Rates of divergence on the two levels are, therefore, judged to be independent of each other; they only vary in unison when the components of the environment to which each is related also vary in parallel.

Divergence in allopatry seems to have proceeded in rates dependent on the environmental differences as each genus (and each level of integration) has perceived them. Divergence in sympatry has been more rapid on the morphological level, possibly because of habitat separation between the closely related congeners.

In a recent article Vawter et al. (1980) have claimed that data from geminate species of fish support the molecular clock hypothesis; they also criticized my suggestion (Lessios, 1979*a*) that the sea urchin data are inconsistent with its predictions. I present arguments as to why, in my opinion, the fish data are not adequate to test this hypothesis, while the conclusions drawn from the sea urchin data should stand.

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