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GENETIC AND MORPHOLOGICAL DIVERGENCE AMONG MORPHOTYPES OF THE ISOPOD *EXCIROLANA* ON THE TWO SIDES OF THE ISTHMUS OF PANAMA

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Abstract.—*Excirolana braziliensis* is a dioecious marine isopod that lives in the high intertidal zone of sandy beaches on both sides of Central and South America. It possesses no larval stage and has only limited means of adult dispersal. Indirect estimates of gene flow have indicated that populations from each beach exchange less than one propagule per generation. Multivariate morphometrics have discovered three morphs of this species in Panama, two of them closely related and found on opposite sides of Central America ("C morph" in the Caribbean and "C′ morph" in the eastern Pacific), the third found predominantly in the eastern Pacific ("P morph"). Though the P and C′ morphs are seldom found on the same beach, they have overlapping latitudinal ranges in the eastern Pacific. A related species, *Excirolana chamensis,* has been described from the Pacific coast of Panama. Each beach contains populations that remain morphologically and genetically stable, but a single drastic change in both isozymes and morphology has been documented. We studied isozymes and multivariate morphology of 10 populations of *E. braziliensis* and of one population of *E. chamensis.* Our objective was to assess the degree of genetic and morphological variation, the correlation of divergence on these two levels of integration, the phylogenetic relationships between morphs, and the possible contributions of low vagility, low gene flow, and occasional extinction and recolonization to the genetic structuring of populations. Genetic distance between the P morph, on one hand, and the other two morphotypes of *E. braziliensis,* on the other, was as high as the distance between *E. braziliensis* and *E. chamensis.* Several lines of evidence agree that *E. chamensis* and the P morph had diverged from other morphs of *E. braziliensis* before the rise of the Panama Isthmus separated the C and C′ forms, and that the P morph constitutes a different species. A high degree of genetic differentiation also exists between populations of the same morph. On the isozyme level, every population can be differentiated from every other on the basis of at least one diagnostically different locus, regardless of geographical distance or morphological affiliation. Morphological and genetic distances between populations are highly correlated. However, despite the high degree of local variation, evolution of *E. braziliensis* as a whole has not been particularly rapid; divergence between the C and C′ morphs isolated for 3 million yr by the Isthmus of Panama is not high by the standard of within-morph differentiation or by comparison with other organisms similarly separated. Alleles that are common in one population may be absent from another of the same morph, yet they appear in a different morph in a separate ocean. The high degree of local differentiation, the exclusive occupation of a beach by one genotype with rare arrival of foreign individuals that cannot interbreed freely with the residents, the genetic stability of populations with infrequent complete replacement by another genetic population, and the sharing by morphs of polymorphisms that are not shared by local populations, all suggest a mode of evolution concentrated in rare episodes of extinction and recolonization, possibly coupled with exceptional events of gene flow that help preserve ancestral variability in both oceans.

Key words.—Gene flow, genetic divergence, morphological divergence, morphotypes, recolonization, variation.

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The continuing debate about the role of gene flow as a cause of intraspecific genetic cohesion (e.g., Mayr 1963, 1970; Ehrlich and Raven 1969; Endler 1973, 1977; Jackson and Pounds 1979; Levin 1979; Ehrlich and White 1980; Pounds and Jackson 1982; Slatkin 1987) owes part of its existence to the difficulty of distinguishing the effects of present-day patterns of genetic exchange from the effects of historical splitting, divergence, and reproductive isolation between populations (Adams 1975; Felsenstein 1982; Larson et al. 1984; Caccone 1985; Caccone and Sbordoni 1987). The difficulty is especially acute in marine organisms, because dispersal is often accomplished during the pelagic larval phase, and because evidence for historical barriers to genetic exchange in the sea is often ambiguous. Organisms with sedentary adults and no planktonic larvae, for which there is independent evidence of historic separation between populations are

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particularly useful in the exploration of the effects of gene flow and the effects of past splitting events on geographic structuring of marine species.

Excirolana braziliensis is a small (mean adult size 3.2 mm), dioecious, marine isopod, that lives in the high intertidal zone of sandy beaches. It is found on both sides of tropical and subtropical America, ranging from the Gulf of California to southern Chile in the eastern Pacific and from the Gulf of Mexico to southern Brazil in the Atlantic. It possesses limited means of dispersal. No larval stage exists. The female broods 4–17 embryos, then releases them into the parental habitat (Dexter 1977). The animals remain buried in the sand during low tide. At high tide, they emerge into the water column to feed, and they may attach to prey, such as live or dead fish, but they usually release after a few seconds (Weinberg pers. obs.) or minutes (Brusca 1980). Studies of the multivariate morphometrics of *E. braziliensis* (Weinberg and Starczak 1988, 1989) have revealed the existence of three morphs, two of them closely related and found on opposite sides of Central America, the third found predominantly in the eastern Pacific but also on the coast of southern Brazil. Weinberg and Starczak (1989) have labeled the Caribbean morph as C, its close counterpart in the eastern Pacific as C′, and the eastern Pacific morph as P. Collections from 43 beaches from the entire range of *E. braziliensis* demonstrated that, with only two exceptions, each beach contains one morph at any given time. Though the reasons for the exclusive occupation of each beach by one morph remain unclear, intensive sampling of three beaches (Weinberg and Starczak 1988) revealed that it is not a sampling artifact. Though they rarely cooccur on the same beach, on a broader geographic scale the Pacific morphs P and C′ are sympatric, in the sense that their ranges coincide in a broad mosaic of no discernable geographic pattern. Bott (1954) and Schuster (1954) have each described a new species of *Excirolana* from the eastern Pacific, but Glynn et al. (1975) and Brusca and Iverson (1985) concluded that these were simple morphological variants and that populations throughout the extended ranges in both oceans belong to *E. braziliensis*. More recently, Brusca and Weinberg (1987) described a new species, *E. chamensis*, from the Pacific coast of Panama.

A 4-yr study of morphological and genetic stability of *E. braziliensis* and *E. chamensis* (Lessios et al. 1994) found that populations in nine localities remained unchanged, whereas both isozymes and morphology in a 10th locality changed so drastically within 2 yr, that replacement of one population by another was the most likely explanation. Such extinction and recolonization events can promote or retard local divergence depending on their frequency and the sources of colonists (Maruyama and Kimura 1980; Slatkin 1977, 1985; Ewens et al. 1987; Barton 1988; Wade and McCauley 1988; Whitlock and McCauley 1990; McCauley 1991; Lande 1992). Estimates based on F_{ST} statistics and private alleles indicate that gene flow between morphs and between local populations of the same morph is low; the restriction appears to be imposed not just by the limited dispersal ability of isopods but also by reduced probability that the migrants can inject their genes into local populations (Lessios and Weinberg 1993). This reproductive isolation between the P and C′ morphs appears strong enough to justify assigning them to separate species.

The separation of Caribbean and eastern Pacific populations by the rise of the Central American Isthmus 3.0 to 3.5 mya (Saito 1976; Keigwin 1978, 1982; Coates et al. 1992) provides a fixed historical point at which gene flow between populations was severed. The existence of distinct morphotypes in *E. braziliensis* and of a closely related species of *Excirolana* also provides points of possible historical branching. Here we combine electrophoretic and morphological evidence from *E. braziliensis* and *E. chamensis* to ask the following: (1) How much genetic differentiation is there within and between morphs and between recognized species of *Excirolana* in the two oceans? (2) Is there a relationship between morphological and isozymic differentiation? (3) What is the probable phylogenetic relationship between morphs of *E. braziliensis*? (4) Based on the assessments of differentiation, gene flow, and genetic stability of the populations, what can be said about the probable mode of evolution in *Excirolana*?

Materials and Methods

Excirolana braziliensis was sampled for electrophoretic and morphological analyses at three localities on the Atlantic and seven localities on the Pacific coast of Panama. *Excirolana chamensis* was collected at Punta Chame, in the Bay of Panama (fig. 1). In all but two beaches, duplicate samples were obtained, one between February 4 and 26, 1986, and one between Septem-

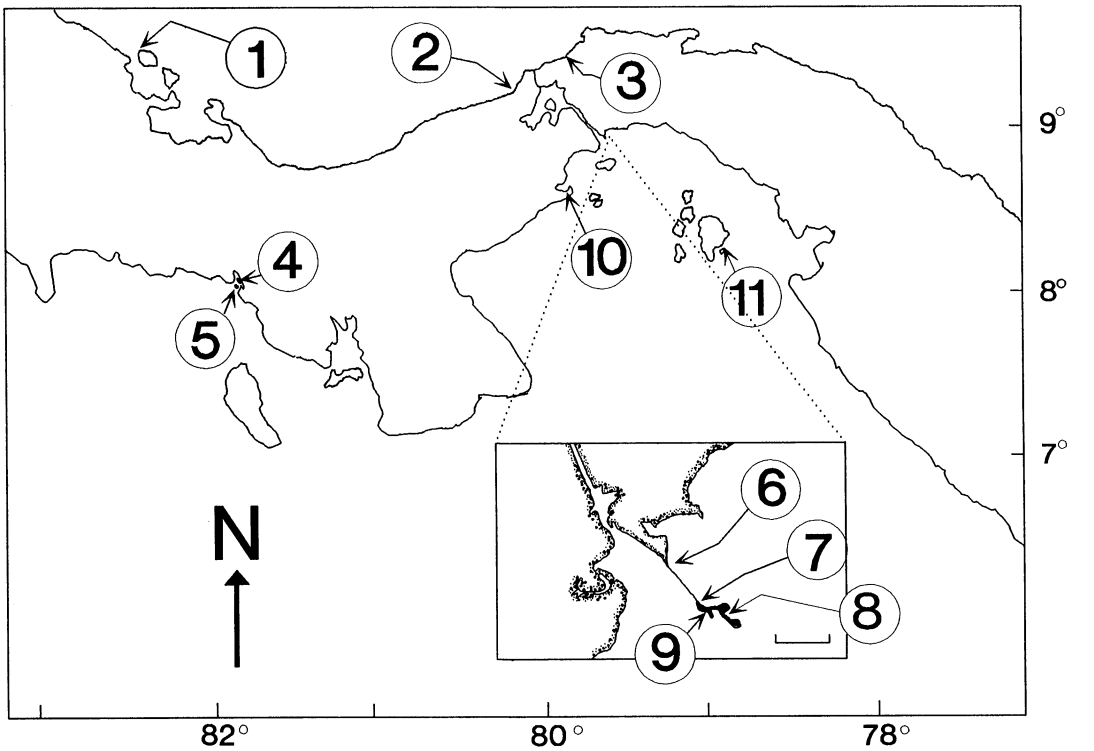

FIG. 1. Locations at which isopods were sampled. Morphotype of *Excirolana braziliensis* at each beach is given in parentheses. 1, Bocas del Toro (C); 2, Shimmey Beach (C); 3, Maria Chiquita (C); 4, Isla Porcada (P); 5, Isla Adentro (C'); 6, Causeway Beach (P); 7, Lab Beach (P); 8, Perico (P); 9, Isla Culebra (C'); 10, Punta Chame (*Excirolana chamensis*); 11, Santelmo (C' and P). Size of scale bar in insert is 2 km.

ber 17 and October 31, 1988. Isopods were collected by sieving (0.5 mm hole size) the uppermost 5-cm layer of sand from the high intertidal zone and transported to the laboratory in plastic bags full of moist sand. Individuals were frozen in liquid nitrogen for electrophoresis or preserved in 95% ethanol for morphometric analysis.

Morphometrics.—Eight characters (body length, body width, eye diameter, rostrum width, length of flagellum on antennule, length of flagellum on antenna, length of last peduncular article on antenna, and length of uropodal exopod) were measured on an approximately equal number of males and females in each of the two sampled years; sample sizes ranged from 10 to 34 per beach (Lessios et al. 1994). An earlier sample, collected between 1984 and 1985 (Lessios et al. 1994) was not used for the present study because it was not accompanied by a genetic sample. Methods of measurement and a discussion of the characters are given in Weinberg and Starczak (1988, 1989). Measurements from each individual were converted to their natural logarithm to stabilize their variances, then adjusted for size using Burnaby's (1966) method as recommended by Rohlf and Bookstein (1987). Principal component analysis performed on these adjusted data revealed that, as found previously (Weinberg and Starczak 1989), the first principal component explained most of the variation (56.82% in the present case). We used the scores of the first principal component, after removal of two outliers (see Lessios et al. 1994), to calculate Mahalanobis generalized distance in a pairwise fashion between all populations. The first principal component represents shape because the size variation is removed by the Burnaby adjustment.

Electrophoresis.—Each animal was homogenized in 200 μL of 10-mM-Tris-0.6-mM-EDTA-HCl buffer, pH 7.0. The homogenates were run in 11% horizontal starch gels (Otto Hiller Lot 392) in buffers listed in table 1. Of 53 enzyme assays tried, the following gave scorable results (designation of presumptive loci in parentheses): Alkaline phosphatase (*Alkph-1* and *Alkph-2*), Es-

terases (*Est-1* and *Est-2*), N-Acetyl-β-glucosaminase (*βGa*), Fructokinase (*Fk*), Fumarate dehydrogenase (*Fum*), Glutamate-oxaloacetate transaminase (*Got*), Glutamic dehydrogenase (*Glutdh*), α-Glycerophosphate dehydrogenase (*αGlyphdh*), Mannose-6-phosphate isomerase (*M6pi*), NAD^+ dependent Malate dehydrogenase (*Mdh-1* and *Mdh-2*), L-Leucyl-L-Tyrosine peptidase (*Peplt*), Phosphoglucose isomerase (*Pgi*), Phosphoglucomutase (*Pgm*), Triosephosphate isomerase (*Tpi*). These enzymes provided a total of 17 scorable loci (table 1). Staining mixtures were those of Ayala et al. (1972) and of Aebersold et al. (1987).

Alleles were standardized between years and between populations by running frozen individuals collected in 1986 in all gels containing individuals collected in 1988 and by including individuals from various populations in the same gel. Because of the high degree of monomorphism within each population (see Lessios and Weinberg 1993), this standardization was adequate. However, the small size of the animals resulted in limited sample volume, which, in turn, prevented the reassaying of individuals with suspected different genotypes side-by-side. Because of this limitation, the approach taken in scoring was the most conservative one possible, that is, every doubtful case was scored so as to decrease apparent variability. Null alleles had to be postulated in *Alkph-2* at Isla Adentro, Isla Culebra, and Punta Chame, and in *Est-1* at Shimmey Beach and Isla Porcada. The majority of individuals from these populations showed no activity in the region of the respective locus (as determined from results from other populations) even though they possessed high activity in *Alkph-1* or *Est-2*. In enzymes with a single locus, no null alleles could be deduced, as there was no way to distinguish lack of activity caused by dilution or denaturation from lack of activity caused by a silenced locus. Every individual at Punta Chame was scored in *Est-1* as an apparent heterozygote between alleles $Est\text{-}1^e$ and $Est\text{-}1^g$. Though this genetic interpretation is almost certainly wrong, it is more conservative in calculating divergence between populations than the postulation of an isolocus with alleles that differ between Punta Chame and all other populations, or of an additional locus with null alleles in all other populations. For various reasons, it was not possible to obtain data from one sample of a particular population for 6 out of the 17 loci. All analyses of the data have either been limited to the 11 loci that were sampled in common for all populations, or they have been calculated twice, once from the common loci, and once from all available loci.

TABLE 1. Enzymes assayed, electrophoretic conditions used, and number of loci scored. See text for full name of enzymes. Buffer designations: A, Discontinuous 0.076 M Tris-Citrate, pH 8.65; B, 0.023 M Tris-Citrate, pH 8.00; C, Discontinuous 0.046 M Tris-Citrate-Borate-LiOH, pH 8.30; D, 0.214 M Phosphate-Citrate, pH 7.00.

Enzyme	Buffer	Voltage (V)	No. of loci
ALKPH	A	200	2
EST	C	250	2
FK	B	75	1
FUM	B	75	1
βGA	D	95*	1
GOT	B	75	1
GLUTDH	B	75	1
αGLYPHDH	D	95*	1
M6PI	A	200	1
MDH	B	75	2
PEPLT	C	250	1
PGI	B	75	1
PGM	C	250	1
TPI	D	95*	1
Total number of loci			17

* In contrast to the other three buffers, in which voltage was held constant, in this buffer current was constant at 150 mA.

Given the temporal stability of most populations in both genetic constitution and morphology (Lessios et al. 1994), we considered it justifiable to calculate genetic and morphological distance values between populations by pooling the 1986 and 1988 data from all localities, except Isla Porcada. At Isla Porcada we saw statistically significant changes in isozymes and in morphology from 1986 to 1988. We have, therefore, treated the 1986 and 1988 Isla Porcada samples as though they came from different populations.

RESULTS

Morphological Differentiation

Mahalanobis distances, calculated from the first principal component of the morphological measurements between all populations, are shown in table 2. They ranged from 0 to 187. An UPGMA (Sneath and Sokal 1973, p. 230) cluster diagram based on Mahalanobis distances (fig. 2) confirms previous conclusions (Weinberg and Starczak 1989) in that there are two major clusters, one encompassing the Caribbean populations (morphological type C) and the Pacific ones with mor-

TABLE 2. Average of Nei's (1978) unbiased genetic distance ($\hat{D}$), jackknifed across loci according to Mueller and Ayala's (1982) procedure (below the diagonal) and Mahalanobis generalized distance (D^2) (above the diagonal) between all populations studied. The morphological type to which individuals from each beach belong (according to Weinberg and Starczak 1989) is indicated above beach name (P, Pacific type; C, Caribbean type, C', Caribbean-like type, *E. cham., Excirolana chamensis*). Data from 1986 and 1988 were pooled for all populations for which they were available, except for the one from Is. Porcada in which there is reason to believe that there was replacement of populations between years (see text). Nei's $\hat{D}$ values without parentheses indicate those based on comparisons of all available loci (11–17). Nei's $\hat{D}$ values in parentheses indicate those based on the 11 loci that were sampled in all populations.

	Atlantic			Pacific								
	C			C'		P					P & C'	*E. cham.*
	Bocas del Toro	Maria Chiquita	Shimmey Beach	Is. Adentro	Is. Culebra	Causeway Beach	Lab Beach	Is. Porcada 1986	Is. Porcada 1988	Perico	Santelmo P & C'	Pta. Chame
Bocas del Toro C	—	1.245	1.144	15.317	5.064	59.706	56.649	43.720	52.919	34.455	0.003	176.380
Maria Chiquita C	0.042 (0.001)	—	0.0001	6.327	1.553	56.045	60.703	45.790	56.905	40.291	0.093	154.579
Shimmey Beach C	0.056 (0.001)	0.069 (0.001)	—	5.914	1.488	50.921	56.933	42.032	52.880	37.826	0.086	140.949
Is. Adentro C'	0.237 (0.085)	0.186 (0.088)	0.220 (0.087)	—	0.841	92.950	103.542	75.168	83.458	67.525	1.151	187.292
Is. Culebra C'	0.235 (0.087)	0.184 (0.091)	0.225 (0.092)	0.062 (0.001)	—	48.781	60.743	43.808	55.909	43.844	0.616	121.143
Causeway Beach P	0.791 (0.725)	0.696 (0.739)	0.758 (0.741)	0.577 (0.748)	0.568 (0.723)	—	0.137	0.061	1.192	0.034	3.159	35.737
Lab Beach P	0.810 (0.730)	0.823 (0.744)	0.824 (0.746)	0.712 (0.753)	0.699 (0.727)	0.061 (0.000)	—	0.339	0.726	0.249	5.262	31.329
Is. Porcada 1986 P	0.666 (0.587)	0.663 (0.593)	0.658 (0.597)	0.683 (0.600)	0.674 (0.588)	0.164 (0.080)	0.075 (0.082)	—	1.524	0.000	3.193	29.976
Is. Porcada 1988 P	0.700 (0.479)	0.710 (0.479)	0.607 (0.488)	0.598 (0.482)	0.595 (0.480)	0.362 (0.225)	0.376 (0.231)	0.116 (0.116)	—	1.379	5.486	13.494
Perico P	0.897 (0.927)	0.914 (0.941)	0.929 (0.953)	0.778 (0.948)	0.767 (0.929)	0.066 (0.091)	0.066 (0.092)	0.179 (0.179)	0.421 (0.286)	—	4.234	25.481
San Telmo P & C'	0.061 (0.021)	0.076 (0.024)	0.090 (0.024)	0.180 (0.052)	0.100 (0.048)	0.704 (0.647)	0.728 (0.652)	0.551 (0.551)	0.713 (0.447)	0.875 (0.847)	—	11.333
Pta. Chame *E. cham*	0.645 (0.558)	0.550 (0.558)	0.661 (0.571)	0.450 (0.423)	0.496 (0.421)	0.544 (0.411)	0.679 (0.417)	0.366 (0.281)	0.456 (0.110)	0.626 (0.426)	0.559 (0.484)	—

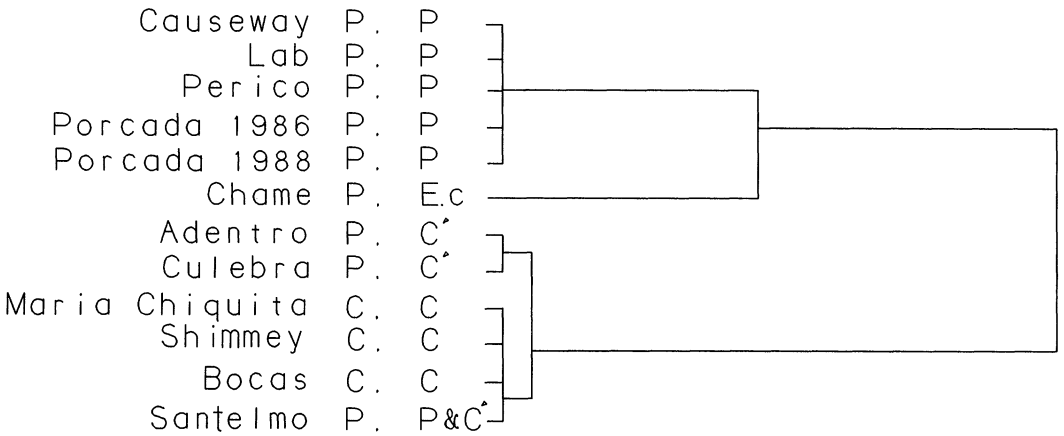

FIG. 2. UPGMA clustering on all populations on the basis of Mahalanobis distance D^2 calculated from the first principal component. The first letter following the name of each locality indicates the ocean in which it is found (P, eastern Pacific; C, Caribbean), the second letter indicates the morphological type to which it belongs (P, Pacific type; C, Caribbean type; C', Caribbean-like type; E.c, *Excirolana chamensis*). Data from 1986 and 1988 were pooled for all populations, except for the one from Is. Porcada in which there was reason to believe that there was replacement of populations between years (see the text).

phological type C', and another that contains the other Pacific morph, P. The P morph of *Excirolana braziliensis* is more closely aligned to *Excirolana chamensis* than it is to the C and C' morphs. Santelmo, one of the two localities (out of 43 studied by Weinberg and Starczak 1989) known to include two morphs, clusters closest to the C morph. Though the clustering with morph C suggests that the majority of individuals at Santelmo are more similar to Caribbean *E. braziliensis* than to other populations of C' morphotype in the eastern Pacific, the issue should be considered as unresolved for two reasons. (1) The multivariate bimodality of this population violates the assumptions on which Mahalanobis distance is based. (2) The mean Mahalanobis distance between Santelmo and the C' populations (0.883) is only slightly larger (considering the 0–187 range of observed values) than the mean distance between Santelmo and the Caribbean populations (0.061).

Genetic Differentiation

Gene frequencies of each population are presented in Lessios and Weinberg (1993). Table 2 shows Nei's unbiased genetic distances (Nei 1978) between all populations. They ranged from 0.042 to 0.929 when calculated on the basis of all available loci and from 0.000 to 0.953 when calculated on the basis of the 11 loci that were common to all comparisons. Interestingly, the highest values were obtained in comparisons between populations of the P and the C morph of *E. braziliensis,* not, as might have been expected, between populations of *E. chamensis* and *E. braziliensis.* The genetic distance between the two samples from Isla Porcada is larger than any distance between populations of the same morph, except for comparisons that involve Isla Porcada itself. Thus, our belief that the two samples from this locality represent different populations is supported by comparison to geographical variation, as well as by statistical comparisons over time (see Lessios et al. 1994).

UPGMA clustering of Nei's genetic distances was performed on the basis of the 11 common loci and on the basis of all available loci (fig. 3). The two cluster diagrams differ only in details. In both instances, all populations of each morph of *E. braziliensis* cluster together. The P morph is highly differentiated from the other two morphs of *E. braziliensis*; *E. chamensis* appears as an outgroup of the P morph, whereas the two C' populations form one cluster, which genetically resembles the C morph. Both clustering diagrams from Nei's distances (fig. 3) are very similar to the clustering diagram from Mahalanobis distances (fig. 2). Thus, genetic discontinuities between morphs parallel closely morphological discontinuities.

We used Mueller and Ayala's (1982) jackknifing procedure and a program written specifically for this purpose (Lessios 1990) to calculate jackknifed means, variances and covariances of

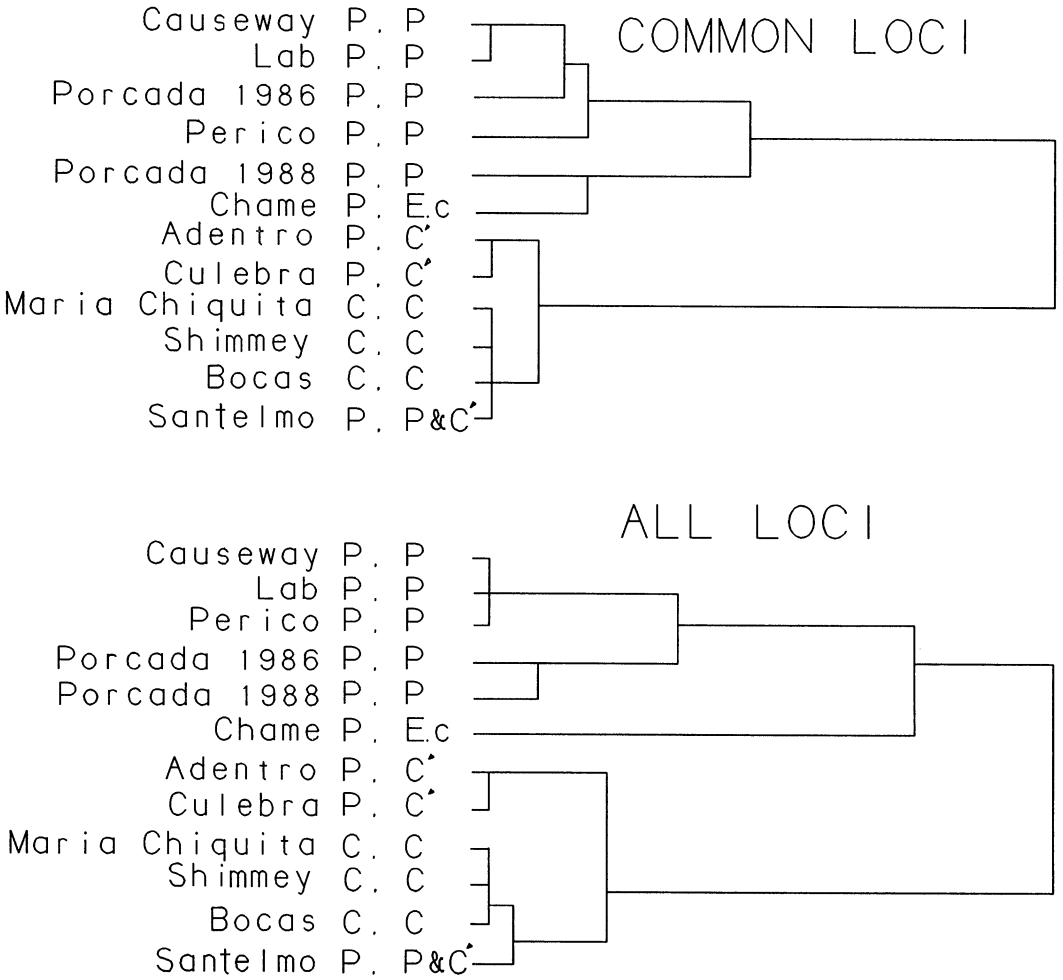

FIG. 3. UPGMA clustering of all populations on the basis of Nei's $\hat{D}$ as calculated by loci sampled in common in all populations (top), and all loci available for each pairwise comparison (bottom). First letter following each locality indicates the ocean in which it is found, the second letter indicates the morphological type to which it belongs. Codes are the same as in figure 2. Data from 1986 and 1988 were pooled for all populations for which they were available, except for the one from Is. Porcada in which there was reason to believe there was replacement of populations between years (see the text).

genetic distances between populations, morphs, and species in the two oceans. We employed the calculated statistics to answer two questions. (1) Which morphs are significantly different? (2) Which pairwise genetic distances between morphs are significantly different? In these comparisons, Santelmo was treated as a separate group.

Following the rationale of Mueller and Ayala (1982) (see also Pamilo 1990), we considered two morphs as significantly different from each other when the average distance between morphs minus the average pooled within-morph distance was significantly different from 0. Because the number of loci sampled in common in all populations was small, a comparison of within- to between-morph distances limited to them showed that only *E. chamensis* was significantly different from the Caribbean morph of *E. braziliensis* (table 3). However, when all available loci were included, the following patterns emerged (table 3): *E. chamensis* was significantly different from all morphs of *E. braziliensis* with which it could be compared. The P morph was also significantly different from all other morphs. However, the C morph in the Atlantic and the C′ morph in the Pacific are not significantly different from each

TABLE 3. Average jackknifed Nei's unbiased distances within morphs (along the diagonal) and between morphs (below the diagonal) of *Excirolana brazilensis,* and *E. chamensis.* Values in parentheses are calculated from the 11 loci common to all comparisons. Significance refers to comparisons of mean intermorph distance to mean intramorph distance in the manner of Mueller and Ayala (1982). Significance of comparison between Santelmo and Chame cannot be determined because each group contains one population. NS, not significant.

	C	C′	P	Santelmo
Caribbean (C)	0.056 (0.001)			
Caribbean-like (C′)	0.215 NS (0.088 NS)	0.062 (0.001)		
Pacific (P)	0.763* (0.670 NS)	0.665* (0.698 NS)	0.189 (0.138)	
Santelmo	0.076 NS (0.023 NS)	0.140 NS (0.050 NS)	0.714* (0.629 NS)	— —
E. chamensis	0.619* (0.562*)	0.473* (0.422 NS)	0.534* (0.329 NS)	0.559— (0.484—)

* $P < 0.05$.

other. Santelmo, though in cluster diagrams appears more closely aligned to the C morph than to the C′ morph (fig. 3), was not significantly different from either.

Jackknifed comparisons of intermorph distances (table 4) were used to determine the genetic affinities of the morphs. Though the pairwise genetic distances between the P morph and the three other morphotypes of *E. braziliensis* were larger than the distances between any of them and *E. chamensis* (table 3), these differences in magnitude were not significant (table 4). Thus, the Pacific morph differed from all other morphotypes of *E. braziliensis* as much as (but no more than) they differ from a recognized separate species. More generally, the multiple comparisons between genetic distances supported the statistical reality of the two major clusters in the UPGMA diagram (fig. 3). A "Pacific branch" exists that includes the P morph of *E. braziliensis* and *E. chamensis,* and there is a "Caribbean-like branch," which includes the true Caribbean forms, plus the C′ morphotype and the mixed population at Santelmo. Genetic distances between morphs within each of these branches were not significantly different from each other.

Nei's *D* is a summary index, useful in quantifying electrophoretic divergence, but not in describing the qualitative genetic differences between populations. Given the large differentiation seen in these isopods, it is interesting to assess how genetically distinct each population is from all others and how many loci help define this distinction. We used the method of Ayala and Powell (1972) to determine the number of loci that were diagnostic between morphs and between populations within morphs. Though our aim is not to determine diagnostic characters for taxonomic purposes, this is a useful means of describing the locus-by-locus differences in these populations. Its results also provide the foundation of our method of using genetic markers for distinguishing individuals that may be migrants between populations (see Lessios and Weinberg 1993).

TABLE 4. Results of multiple pairwise comparisons between average Nei's unbiased intermorph distances. Average distances (see table 3) are arranged from smallest to largest. Distances connected with a line are not significantly ($P = 0.05$) different. Distance between the Santelmo population of *Excirolana braziliensis* and *Excirolana chamensis* is not shown because a single population was sampled from each of these "morphs."

Genetic distance	All loci	Common loci
Caribbean (C) vs. Santelmo (ST)		
Caribbean-like (C′) vs. Santelmo (ST)		
Caribbean (C) vs. Caribbean-like (C′)		
Caribbean-like (C′) vs. *E. chamensis* (*E. cham*)		
Pacific (P) vs. *E. chamensis* (*E. cham*)		
Caribbean (C) vs. *E. chamensis* (*E. cham*)		
Caribbean-like (C′) vs. Pacific (P)		
Pacific (P) vs. Santelmo (ST)		
Caribbean (C) vs. Pacific (P)		

TABLE 5. Diagnostic loci sensu Ayala and Powell (1972) that permit discrimination at the 90% level or better between the morphs of *Excirolana braziliensis* defined by Weinberg and Starzak (1988, 1989) and between *E. braziliensis* and *Excirolona chamensis*. The probability of assignment of each individual to the correct morph on the basis of each diagnostic locus is given in parentheses. All available data from all the populations belonging to each morph were pooled.

	Caribbean (C) morph		Caribbean-like (C') morph		Pacific (P) morph		Santelmo	
Caribbean-like (C') morph	*αGlyphdh*	(0.92)	—					
	Alkph-2	(0.99)						
	Est-1	(1.00)						
	Est-2	(1.00)						
Pacific (P) morph	*αGlyphdh*	(0.97)	*αGlyphdh*	(0.97)	—			
	Alkph-1	(1.00)	*Alkph-1*	(1.00)				
	Alkph-2	(1.00)	*Alkph-2*	(1.00)				
	Est-1	(0.97)	*Est-1*	(1.00)				
	Est-2	(1.00)	*Glutdh*	(1.00)				
	Glutdh	(1.00)	*Got*	(1.00)				
	Got	(1.00)	*M6pi*	(1.00)				
	M6pi	(1.00)	*Mdh-1*	(0.99)				
	Mdh-1	(1.00)	*Pgi*	(0.96)				
	Pgi	(0.95)						
Santelmo	*Est-1*	(1.00)	*Alkph-3*	(0.91)	*Alkph-1*	(1.00)	—	
			Est-2	(1.00)	*Alkph-2*	(1.00)		
					Est-1	(1.00)		
					Est-2	(1.00)		
					Glutdh	(1.00)		
					Got	(0.98)		
					M6pi	(1.00)		
					Mdh-1	(0.99)		
					Pgi	(0.96)		
E. chamensis	*αGlyphdh*	(0.93)	*Alkph-1*	(1.00)	*αGlyphdh*	(0.98)	*Alkph-1*	(1.00)
	Alkph-1	(1.00)	*βGa*	(1.00)	*Alkph-2*	(1.00)	*Alkph-2*	(0.94)
	Alkph-2	(1.00)	*Est-2*	(1.00)	*βGa*	(1.00)	*βGa*	(1.00)
	βGa	(1.00)	*Got*	(1.00)	*Est-1*	(1.00)	*nEst-1*	(1.00)
	Est-1	(0.91)	*M6pi*	(1.00)	*Est-2*	(1.00)	*Est-2*	(1.00)
	Est-2	(1.00)	*Pgm*	(0.99)	*Glutdh*	(0.99)	*Got*	(0.98)
	Got	(1.00)			*Mdh-1*	(0.99)	*M6pi*	(1.00)
	M6pi	(1.00)			*Pgi*	(0.96)	*Pgm*	(1.00)
	Pgm	(0.99)			*Pgm*	(0.98)		

As table 5 indicates, there was no one locus that could distinguish between all morphs; however individuals belonging to each morphotype were distinct in 1 to 10 loci. Even though the population at Santelmo was treated as a separate morph not because of its morphological distinctiveness, but because it contains individuals from two morphs, its individuals are genetically distinct from all other morphs. Thirteen of the 17 loci were diagnostic in at least one pairwise comparison between morphs. Only *Fk, Fum, Mdh-2,* and *Peplt* did not differentiate between any of the morphs (see Lessios and Weinberg 1993). This is a remarkable degree of discrimination between populations that systematists, after careful examination, defined as conspecific (Glynn et al. 1975; R. Brusca pers. comm. 1988).

Even more remarkable is that this level of discrimination is extended to comparisons of populations belonging to the same morph (table 6). Populations at Lab Beach and Perico both belong to the P morph, are separated by less than 1 km of coastline, yet they had completely different alleles in *Pgm*. Causeway and Lab are approximately 2 km apart but showed no overlap in *αGlyphdh*. Even the 1986 collection at Isla Porcada differed from the 1988 collection in *Mdh-1*. Thus, there was no population among the 12 we sampled that could not be unambiguously told apart from all others on the basis of at least 1 of 17 loci. These results suggest that every local population of *E. braziliensis* on both the Pacific and Atlantic coasts of Panama is genetically distinct from every other population, regardless of

TABLE 6. Diagnostic loci sensu Ayala and Powell (1972) that permit discrimination at the 90% level or better between populations of the same morphological type of *Excirolana braziliensis* (as defined by Weinberg and Starzak 1988, 1989). The probability of assignment of each individual to the correct population on the basis of each diagnostic locus is given in parentheses. Data from 1986 and 1988 from each population were pooled. Santelmo is compared with populations of both the C′ and P morphs because it contains individuals of both.

Caribbean (C) morph					Caribbean-like (C′) morph				
	Bocas del Toro		Maria Chiquita			Is. Adentro		Is. Culebra	
Maria Chiquita	*αGlyphdh*	(0.98)	—		Is. Culebra	*Est-1*	(1.00)		
Shimmey Beach	*Est-1*	(1.00)	*Est-1*	(1.00)	Santelmo	*Alkph-2*	(0.90)	*Alkph-2*	(0.92)
						Est-1	(1.00)	*Est-2*	(1.00)
						Est-2	(1.00)		

Pacific (P) morph										
	Causeway Beach		Lab Beach		Is. Porcada 1986		Is. Porcada 1988		Perico	
Lab Beach	*αGlyphdh*	(1.00)								
Is. Porcada 1986	*αGlyphdh*	(1.00)	*Pgi*	(1.00)						
Is. Porcada 1988	*Est-1*	(1.00)	*Est-1*	(1.00)	*Mdh-1*	(1.00)				
	Glutdh	(1.00)	*Glutdh*	(1.00)						
	Pgi	(1.00)	*Mdh-1*	(1.00)						
	Tpi	(1.00)	*Pgi*	(1.00)						
			Tpi	(1.00)						
Perico	*Pgm*	(1.00)	*Pgm*	(1.00)	*Pgi*	(1.00)	*Est-1*	(1.00)		
					Pgm	(1.00)	*Glutdh*	(1.00)		
							Mdh-1	(1.00)		
Santelmo	*Alkph-1*	(1.00)	*Alkph-1*	(1.00)	*Alkph-1*		*Alkph-1*		*Alkph-1*	(1.00)
	Alkph-2	(1.00)	*Alkph-2*	(1.00)		(1.00)		(1.00)	*Alkph-2*	(1.00)
	Est-1	(1.00)	*Est-1*	(1.00)	*Alkph-2*		*Alkph-2*		*Est-1*	(1.00)
	Est-2	(1.00)	*Est-2*	(1.00)		(1.00)		(1.00)	*Est-2*	(1.00)
	Glutdh	(0.99)	*Glutdh*	(1.00)	*Got*	(0.98)	*Est-1*	(1.00)	*Glutdh*	(1.00)
	Got	(0.98)	*Got*	(0.98)	*M6pi*	(1.00)	*Est-2*	(1.00)	*Got*	(0.99)
	M6pi	(1.00)	*M6pi*	(1.00)	*Mdh-1*	(1.00)	*Glutdh*	(0.99)	*M6pi*	(1.00)
	Mdh-1	(1.00)	*Mdh-1*	(1.00)			*M6pi*	(1.00)	*Mdh-1*	(1.00)
	Pgi	(1.00)	*Pgi*	(1.00)			*Tpi*	(1.00)		

geographical distance or morphological affiliation.

Correlation between Morphological and Genetic Divergence

Cluster diagrams generated on the basis of Mahalanobis distance and Nei's distance are similar, suggesting an equivalent degree of divergence on the two levels of integration. We quantified this relationship by calculating correlation coefficients between Nei's D and Mahalanobis D^2 for all populations, regardless of morph affiliation (fig. 4). Because of the interdependence of pairwise divergence measures, we determined the significance of Kendall's rank correlation coefficient, τ, using Mantel's (1967) procedure. A null distribution for the correlation coefficient between the two distance measures was obtained by computing the τ statistic after randomly permuting the values in one of the distance matrices 1000 times. The procedure was carried out separately for common loci and all loci. The correlation coefficients relating genetic and morphological distances (Kendall's $\tau = 0.502$ for common loci, $\tau = 0.474$ for all loci) were highly significant ($P < 0.001$, in both cases).

One can always obtain a significant correlation between measures of divergence based on different sets of characters by including comparisons with a highly divergent taxon, but in the case of these isopods the inclusion of *E. chamensis* actually detracted from the tightness of the correlation (fig. 4). When we excluded this species, the correlation coefficients were higher ($\tau = 0.535$ for common loci, $\tau = 0.523$ for all loci, $P < 0.001$ in both cases). The good agree-

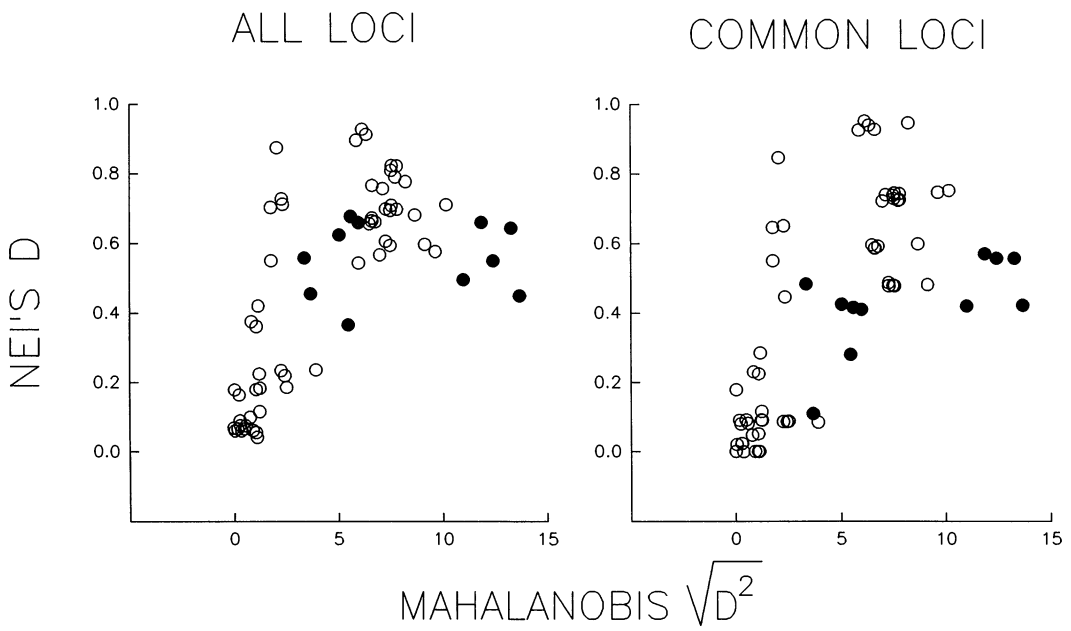

FIG. 4. Correlation between Nei's genetic distance $\hat{D}$ (based on all available loci for each comparison and the 11 loci sampled in common in all populations) and Mahalanobis generalized distance $\sqrt{D^2}$. Open circles, distance measures between populations of *Excirolana braziliensis*; closed circles, distance measures between *E. braziliensis* and *E. chamensis*.

ment between clustering performed on the basis of isozyme and morphology and the tight correlation between genetic and morphological distances leave no doubt that morphological divergence reflects genetic differentiation.

Phylogeny

A phylogenetic hypothesis for *Excirolana* was needed to distinguish variation between populations that resulted from cladogenesis from variation that was caused by patterns of gene flow. The cluster diagrams in figures 2 and 3 would have also been phylogenetic trees if rates of evolution of the characters on which they were constructed were constant through time, an unsafe assumption for both morphological and isozyme characters. We have, therefore, constructed a distance Wagner tree, using the MANAD procedure of Swofford and Berlocher (1987) and Swofford's FREQPARS computer program. The objections of Crother (1990) to the use of gene frequencies in phylogeny reconstruction have been addressed (Lessios et al. 1994). Our cluster analyses and assessment of reproductive isolation (Lessios and Weinberg 1993) indicate that all morphs of *E. braziliensis* do not necessarily belong to the same species. We, therefore, cannot assume that *E. chamensis* is a certain outgroup to all other populations. Thus, the parsimony network (fig. 5) could not be rooted. However, the relationships between populations suggested by the unrooted network agreed well with the cluster diagram of Nei's distances and thus with the morphological subdivisions as well. All Atlantic populations could be derived from one hypothetical ancestor. The Isthmus of Panama separated the C morph from the C′ morph, with Santelmo, as previously, coming out closest to the Caribbean populations. The two C′ populations required the postulation of two additional hypothetical ancestors to be derived from the Caribbean populations. *Excirolana chamensis* was more closely related to the P and C′ morphs than they were related to each other. The three populations with the P morphotype that are geographically closest to each other (fig. 1), Causeway, Lab, and Perico, were related through two hypothetical ancestors, whereas the two Porcada populations required additional nodes to be connected to the rest of the Pacific populations. Interestingly, the two Porcada populations required the postulation of two hypothetical ancestors to be derived from each other. Thus, a parsimony analysis of gene frequencies was in complete agreement with the

distance analysis of both gene frequencies and morphology as to the phylogenetic relationships between populations.

Discussion

Phylogenetic Relationships between Morphs

Phenograms based on distance analyses of isozymes and morphology agree as to the relationships of the three morphs of *Excirolana braziliensis* with respect to each other and with respect to *Excirolana chamensis.* The parsimony analysis of the isozyme data also agrees with the distance analyses. There is no a priori reason to expect magnitudes of morphological and isozyme divergence between populations to be highly correlated with each other, particularly on the intraspecific level, and many studies have found discrepancies between the two (e.g., Johnson 1974; Avise et al. 1975; Gould et al. 1975; Jones et al. 1980; Lessios 1981; Bell et al. 1982; Johnson et al. 1987; Kyriakopoulou-Sklavounou et al. 1991). The agreement of divergence on two levels of integration in *Excirolana* suggests that they both reflect underlying substantial genetic differentiation, which could be the result of ancient separation of lineages or of rapid evolution. Our isozyme phylogeny lends support to previous conclusions about the phylogenetic relationships of the three morphs of *E. braziliensis,* reached on the basis of multivariate morphometrics alone (Weinberg and Starczak 1989): the P morph split from other morphs of *E. braziliensis* long before C and C′ morphs were separated on either side of the Isthmus of Panama. Though the sequence of splitting of the morphs seems straightforward, the actual timing of the splitting events is less so.

Weinberg and Starczak (1988, 1989) considered the possibility that P and C morphs were a "geminate pair" (Jordan 1908) created by the rise of the Isthmus of Panama, and that the C′ morph was recently introduced into the eastern Pacific as the result of activities related to the construction and operation of the Panama Canal during the last 90 yr. Because of the magnitude of morphological divergence, Weinberg and Starczak (1989) rejected this hypothesis in favor of the alternative, that the Pliocene severance of seawater connections between Caribbean and eastern Pacific separated the C′ and C morphs, whereas the P morph is the result of a split that occurred long before the erection of the isthmus. Our results support this conclusion. The average

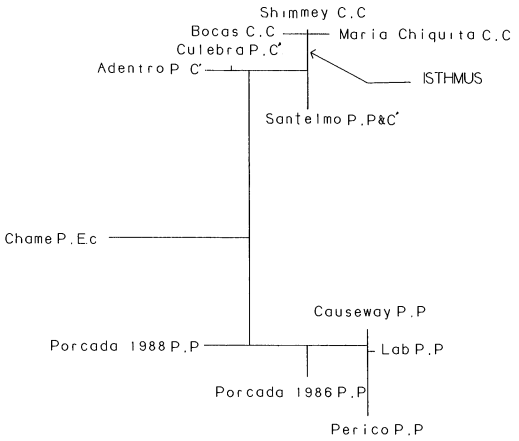

FIG. 5. Unrooted maximum parsimony Wagner tree, generated by the MANAD procedure of Swofford and Berlocher (1987) on the basis of gene freqencies of the 11 loci sampled in common in all populations. Branch length is proportional to differentiation between populations. The first letter following each locality indicates the ocean in which is it found, the second letter indicates the morphological type to which it belongs. Codes are the same as in figure 2. Data from 1986 and 1988 were pooled for all populations for which they were available, except for the one from Is. Porcada in which there was reason to believe there was replacement of populations between years (see the text).

Nei's genetic distance between the C and C′ morph (without Santelmo) is 0.215, whereas that between the C and P morph is 0.763. The degree of differentiation between C and C′ is congruent with transisthmian electrophoretic differentiation in other groups of organisms, whereas divergence between C and P exceeds any known transisthmian value of Nei's *D.* Sea urchins believed to have been separated by the rise of the Isthmus of Panama display Nei's *D* values that range from 0.026 to 0.549 when calculated on the basis of 18 loci (Lessios 1979, 1981) and from 0.174 to 0.341 when calculated on the basis of 34 loci (Bermingham and Lessios 1993). Geminate species of crabs have analogous values of 0.064 to 0.380 (West 1980). *D* values between fish from the two sides of the isthmus range from 0.105 to 0.266 (Vawter et al. 1980). Because of the wide variation of these values of divergence between members of species pairs, presumably created at the same time, we have questioned the notion that isozyme differentiation proceeds in a clocklike manner (Lessios 1979, 1981; Bermingham and Lessios 1993). However, isozymes, like any other character, are more likely to have diverged the longer lineages have re-

mained separate (Thorpe 1982). Thus, the isozyme data provide additional evidence against the hypothesis of a very recent introduction of the C′ morph into the eastern Pacific.

Given the high morphological and genetic similarity of the Caribbean morph with Santelmo, it may seem more probable that propagules from the other side of the isthmus have invaded this locality in the Perlas Islands. However, we consider such a proposal as unlikely. Transportation of moist sand (the most likely mode of anthropogenic conveyance of the isopod, see Weinberg and Starczak 1989) between the Caribbean coast and a remote and uninhabited island in the south end of the Perlas Archipelago seems very unlikely. Nor is it likely that Santelmo was colonized from some other Pacific population, which was recently introduced from the Caribbean. The only other population found by Weinberg and Starczak (1989) to contain a mixture of morphs was on the coast of Ecuador. Even if Santelmo were of recent Caribbean origin, it is certain that neither it nor a population similar to it could have given rise to other populations of the C′ morph within the last 90 yr. The C′ morph is found as far south as Chile and as far north as Costa Rica with many populations in between (Weinberg and Starczak 1989); it is highly unlikely that an isopod with such limited means of dispersal could have traveled that quickly.

The Specific Status of Populations of Excirolana braziliensis

Though it is possible to root the parsimony network shown in figure 5 in such a way that *E. chamensis* becomes the outgroup of all populations of *E. braziliensis,* the cluster diagrams based on Mahalanobis and Nei's distance suggest that *E. braziliensis,* as currently defined by conventional taxonomic criteria, is a polyphyletic taxon, which includes *E. chamensis.* This apparent paradox would be resolved if it were accepted that the C and C′ morphs belong to a different species than the P morph. Two lines of evidence support this hypothesis. (1) Genetic divergence of the P morph from C and C′ morphs is too high for them to be considered as conspecific. (2) Comparison of gene flow and migration estimates suggest that even though representatives of the P and C′ morphs land on beaches occupied by each other, intermorph hybridization is rare (Lessios and Weinberg 1993).

Excirolana braziliensis shows an exceptional degree of genetic differentiation between populations that taxonomists (Glynn et al. 1975; R. Brusca pers. comm. 1988) consider as conspecific. In a review of the entire literature of isozyme divergence on different taxonomic levels, Thorpe (1982, 1983) has found that 80% of Nei's intraspecific distance values fall below 0.05; the highest intraspecific distance in the data he reviewed was in the neighborhood of 0.300. Since then, Johnson et al. (1984) found Nei's *D* between British and Italian populations of the land snail *Cepaea nemoralis* to be 0.631. Vainola and Varvio (1989) have reported a *D* value of 0.480 between supposedly conspecific European and north American populations of the freshwater amphipod *Pontoporeia affinis.* Thus, the genetic distances between the P and other morphs of *E. braziliensis* (0.568–0.929 when calculated from all loci, 0.723–0.953 when calculated from common loci) are among the largest ever reported between populations believed to belong to the same species. A locus-by-locus analysis also casts doubts on the validity of the current inclusion of all morphs in the same species. As a rule, conspecific populations may differ significantly from each other in gene frequencies, but it is unusual for them to be qualitatively different in the majority of their loci (Ayala 1975; Avise 1976), even in species that lack a dispersal phase in their life history (e.g., Berger 1973; Campbell 1978; Bulnheim and Scholl 1981; Parker et al. 1981; McDonald 1985, 1987, 1991; Grant and Utter 1988). Even in the copepod *Tigriopus californicus,* a species that, like *E. braziliensis,* inhabits the upper intertidal zone with sharp habitat discontinuities separating populations that are genetically differentiated from each other, the differences are in magnitude of allele frequencies rather than in fixation of alternate alleles (Burton et al. 1979; Burton and Feldman 1981; Burton 1986). In the most extreme cases of "intraspecific" divergence, European and American populations of the amphipod *Pontoporeia affinis* are diagnostically different in 32% of their loci (Vainola and Varvio 1989), populations of the mussel *Mytilus edulis* from the North and Baltic Seas differ in 23% of their loci (Vainola and Hvilsom 1991), populations of the barnacle *Chthamalus fissus* from the coasts of California and Panama differ in 46% of their loci (Hedgecock 1979), and populations of another isopod, *Cyathura polita,* from the coasts of Maryland and Georgia are different in 50% of their loci (Parker et al. 1981). In all of these extreme cases, isozyme divergence has been used as evidence for the assignment of

local populations to different species. The P and C morphs of *E. braziliensis* are fixed for alternate alleles in 59% of their loci. Thus, morphs of this species are genetically very different when they are compared with datum sets from other species, whether one uses a summary statistic, or whether the comparison is on the actual genetics.

If we accept the suggestion made by Thorpe (1982) and supported by Nei (1987, p. 245) that a *D* value greater than 0.163 between allopatric populations indicates that they belong to different species, then we should conclude that speciation events separate the P, C, and C′ morphs from each other. Indeed, by that criterion, even the P morph should be subdivided into separate species, because the *D* values between the 1988 sample of Isla Porcada and other P populations exceed this cut-off value. However, Thorpe's suggestion is based on previous empirical data with no theoretical reason for delimiting a species on the basis of an exact degree of divergence. Because *D* values from different species can depend on the loci assayed and the buffer systems used, it is preferable to use an "internal standard" to evaluate relative electrophoretic divergence. *Excirolana chamensis* was included in our study to provide such a standard. Its genetic distances from all morphs of *E. braziliensis* are smaller (and not statistically different) than the genetic distances between the P morph and other morphs of *E. braziliensis.* Thus, by this internal standard, the P morph at least, belongs to a different species.

Genetic divergence between the P and the C′ morphs suggests that they belong to different species, but the conclusive evidence with respect to this question is whether they are reproductively isolated. Lessios and Weinberg (1993) have shown that rates of gene flow between populations of the two morphs are as low as apparent levels of gene flow across the Isthmus of Panama and no larger than the rate of genetic exchange between *E. braziliensis* and *E. chamensis.* Given that populations of the P and C′ morphs are geographically interspersed and that they land into beaches occupied by each other, restrictions of gene flow between them must be caused by reproductive isolation, rather than low dispersal. Assortative mating of the P and C′ morphs (or selection against hybrids) is also indicated by significant deficiencies of heterozygotes between genotypes at Santelmo (Lessios and Weinberg 1993). It is, therefore, highly probable that the P morph belongs to a different species than the other two morphs of *E. braziliensis.* It is also possible that the other two morphs have speciated and that *E. braziliensis* is a complex of many sibling species.

Possible Mode of Evolution in Excirolana braziliensis

Divergence between populations of the same morph is large by the standards of what is expected from closely situated local populations, whereas differentiation between the C and C′ morphs, separated by the Central American Isthmus for three million yr is not unusually high. Indeed, divergence between the C and C′ morphs is not significantly different from divergence between local populations of the same morph. If our conclusion that recent migration through the Panama canal has not occurred is correct, then populations of the same morph in the same ocean are genetically only slightly less isolated from one another than populations separated for 3 million yr by Central America, and their evolution has been essentially independent for a very long time. An ancient separation between local populations with very low gene flow is also compatible with the lack of any parallel variation of different loci across local populations and a general lack of correspondence between geographic and genetic distance (cf. fig. 1 vs. table 2). However, though the absence of a dispersal phase in the life history of *Excirolana* may explain how differences between populations are maintained, it is not an adequate explanation of how the geographic mosaic of highly differentiated populations came to be.

Differentiation in the absence of gene flow is caused by the emergence of new alleles in different populations through mutation and the subsequent local increase of their frequency aided by genetic drift and natural selection. However, as it is unlikely that mutation would lead to the appearance of the same alleles in different populations, new alleles, in the complete absence of gene flow, should be unique to the population in which they arose and to populations that are its direct descendants. To the extent that similar electromorphs are the product of the same gene, the distribution of alleles in the *Excirolana* data is not compatible with such an expected pattern. Rather, an allele present in one population may be absent from another of the same morph, yet it may appear in samples from a different morph in a different ocean. This is true for both common and rare alleles, thus it is unlikely to be entirely

caused by our conservation scoring of electrophoretic gels. For example, *Est-1*^{a} is fixed at Shimmey Beach although absent from the other two Caribbean populations; however, it is also nearly fixed at Isla Porcada in the eastern Pacific (see Lessios and Weinberg 1994). *Est-1*^{f} is fixed in Isla Culebra and Santelmo, absent everywhere else in the Pacific, but present in two populations in the Caribbean. *αGlyphdh*^{c} is present at Maria Chiquita and Shimmey beaches in the Caribbean, but absent from Bocas del Toro in the same ocean; it appears again in some of the samples from the Pacific, including samples from *E. chamensis* but is absent at Lab Beach or Isla Porcada. Cases such as these are the reason that estimates of gene flow from private alleles produced the incongruous result that populations separated by land masses and species barriers appeared by this method to be connected by higher levels of gene flow than populations of the same morph (Lessios and Weinberg 1993). This pattern could be explained by natural selection. However, such an explanation would require the postulation of selective pressures that are similar in different oceans while differing so much between beaches 1 km apart that they lead to fixation of an allele in one population and its complete elimination in another. The pattern could also be explained by multiple invasions from one ocean into the other, but such a scheme is highly implausible, considering the low probability of transportation across the isthmus and of subsequent spread by gene flow along both coasts. A more likely explanation is that in *Excirolana* we see different remnants of ancestral polymorphisms that are accidentally preserved in different populations through infrequent events of allele loss due to extinction and recolonization.

Frequent episodes of extinction and recolonization homogenize the metapopulation species pool (Maruyama and Kimura 1980; Slatkin 1985; Ewens et al. 1987; Barton 1988). However, when these events occur infrequently, and when the propagules are drawn from a single population [the "propagule-pool" model of Slatkin (1977) and Wade and McCauley (1988)], they can exert a strong differentiating effect because of genetic drift (Wright 1940; Selander 1975; Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990; McCauley 1991). Differentiation is enhanced when the number of colonists into empty habitats exceeds the number of migrants transporting genes between extant populations in each generation (Wade and McCauley 1988). *Excirolana braziliensis* meets all the criteria of a species in which local populations could diverge because of extinction and recolonization. It has a low (but measurable) rate of local extinction (Lessios et al. 1994), it possesses the potential to recolonize empty habitats from propagules derived from a single population, and it has low rates of gene flow between existing populations (Lessios and Weinberg 1993). Studies of intertidal sand beaches in tropical America (Dexter 1974, 1977, 1979; Jaramillo 1978) have shown *E. braziliensis* to be abundant in 19 out of 20 beaches examined, which suggests that this isopod rarely becomes extinct locally, or that if it does, it rapidly recolonizes and expands its populations. The single locality where *E. braziliensis* was absent was the remote island of San Andres (Dexter 1974). That the number of potential colonists of this species exceeds the number of individuals transporting genes between existing populations is evident from the excess of estimated migration over gene flow rates (Lessios and Weinberg 1993). Because of a gestation period of 37 d (Dexter 1977), a few propagules of *E. braziliensis* have the potential for successful colonization, which could lead to pronounced founder effects (Mayr 1942, p. 237, 1970, p. 303). These founder events would result in both high interpopulation divergence and low intrapopulation variability, the pattern seen in *E. braziliensis*.

Given the low within-population genetic variability of *E. braziliensis,* most colonists in empty habitats are likely to carry alleles common in the source population. Thus, every extinction and colonization event would not necessarily result in divergence between local populations. Occasionally, however, an allele rare in the source population could be introduced into a newly founded population, and—aided by small population size—become fixed. The resulting large interpopulation differences would then be preserved by the absence of gene flow. Subsequent, slow evolution may complete this differentiation through the accidental loss of the rare allele in the source population. Such a scheme of evolution caused by founder events followed by periods of stability has been put forth by Boileau and Hebert (1991) to explain large genetic differences between local populations of freshwater copepods in North America and could serve as a likely explanation for the high divergence between local populations of *E. braziliensis* as well. However, it raises the question of why common

alleles are preserved for such a long time in the gene pool of metapopulations in both oceans.

If high divergence between local populations is caused by extinctions of one population and recolonization by another, one can ask why all of the ancestral alleles, inherited from stock that predates the rise of the isthmus, have not disappeared, resulting in much higher transisthmian divergence. Explaining the pattern away as a possible artifact of the limited resolving power of electrophoresis would not suffice, because the same electrophoretic techniques have detected high levels of divergence between local populations. One possible answer to the question is that founder effects in each ocean can preserve alleles on their way to extinction in one population by helping them become fixed in a newly established population. Another possible explanation is that in *E. braziliensis* exceptional coexistence and hybridization of genotypes leads to the reintroduction of variation from one population into another. The Santelmo population indicates that though coexistence of genotypes is rare, it does occur. This coexistence, even if transient, may reintroduce some of the ancestral variability into the two populations if reproductive isolation has not formed an absolute barrier and thus restart the entire sequence of accidental loss of alleles through genetic bottlenecks.

In Lessios et al. (1994) we calculated that the probability of extinction of a population of *Excirolana* and recolonization of a given locality is 0.028 per year, or 0.009 per generation. However, this is not an estimate of the number of episodes of rapid evolution that occur in the genus, because most of these events probably involve the replacement of one population by another without any accompanying genetic change. The minimum number of evolutionary episodes that can account for the present-day distribution of gene frequencies between populations, is provided by the parsimony network in figure 5. Three such events, and a transition through only two hypothetical ancestors, are needed to produce the observed gene frequencies at Santelmo from the Caribbean populations we sampled. Four additional events, and a transition through three hypothetical ancestors, are needed to produce the populations of the C′ morph we sampled in the Pacific. Note that if we are correct in assuming that the populations may become differentiated during colonization but remain genetically stable thereafter, these "hypothetical" ancestors need not remain hypothetical. Further sampling could discover populations with gene frequencies that resemble closely the expectations of the parsimony algorithm. Thus, in principle at least, our hypothesis is verifiable, though not necessarily falsifiable.

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