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TEMPORAL VARIATION IN POPULATIONS OF THE MARINE ISOPOD *EXCIROLANA*: HOW STABLE ARE GENE FREQUENCIES AND MORPHOLOGY?

H. A. LESSIOS,[1] JAMES R. WEINBERG,[2] AND VICTORIA R. STARCZAK[3]
[1]*Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama*
[2]*National Marine Fisheries Service, Population Dynamics Branch, 166 Water Street, Woods Hole, Massachusetts 02543*
[3]*Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543*

Abstract.—*Excirolana braziliensis* is a dioecious marine isopod that lives in the high intertidal zone on both sides of tropical America. It lacks a dispersal phase and displays a remarkable degree of genetic divergence even between localities less than 1 km apart. Nine populations of this nominal species from both sides of the Isthmus of Panama and one population of the closely allied species, *Excirolana chamensis*, from the eastern Pacific were studied for 2 yr for allozymic temporal variation in 13 loci and for 3 to 4 yr for morphological variation in nine characters. The genetic and morphological constitution of 9 out of 10 populations remained stable. Allele frequencies at two loci and overall morphology in a tenth beach occupied by *E. braziliensis* changed drastically and significantly between 1986 and 1988. The change in gene frequency is too great to explain by genetic drift occurring during a maximum of 14 generations regardless of assumed effective population size; drift is also unlikely to have caused observed changes in morphology. Selective survival of a previously rare genotype is more plausible but still not probable. The most credible explanation is that the resident population at this locality became extinct and that the beach was recolonized by immigrants from another locality. Such infrequent episodes of extinction and recolonization from a single source may account for the large amount of genetic divergence between local populations of *E. braziliensis*. However, the low probability of large temporal genetic change even in a species such as this, in which gene flow between local demes is limited and generation time is short, suggests that a single sample through time is usually adequate for reconstructing the genetic history of populations.

Key words.—*Excirolana*, extinction, gene frequencies, morphology, recolonization, variation.

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The study of temporal variation in the genetic constitution of populations has the potential for documenting evolutionary processes and for revealing the agents responsible for microevolutionary change. Accordingly, the subject has received wide attention, both theoretical (Lewontin and Krakauer 1973; Templeton 1974; Schaffer et al. 1977; Pamilo and Varvio-Aho 1980; Wilson 1980; Nei and Tajima 1981; Watterson 1982; Pollak 1983; Waples 1989a,b, 1990a,b; Waples and Teel 1990), and empirical (Dobzhansky 1943, 1947; Fisher and Ford 1947; Ehrlich and Mason 1966; Bishop 1969; Tamarin and Krebs 1969; Berger 1971; Gaines and Krebs 1971; Krimbas and Tsakas 1971; Dobzhansky and Ayala 1973; Redfield 1973; Gaines et al. 1978; Koehn and Williams 1978; Kohn and Tamarin 1978; Powers and Place 1978; Steiner 1979; Nygren 1980; Bulnheim and Scholl 1981; Burton and Feldman 1981; Cavener and Clegg 1981; Patton and Feder 1981; Tomiuk and Wohrmann 1981; Mihok et al. 1983; Smith and Patton 1984; Gyllensten 1985; Lessios 1985; McClenaghan et al. 1985; Mueller et al. 1985a,b; DeSalle et al. 1987; Blouw et al. 1988; Daly and Patton 1990; Loxdale and Brookes 1990; Waples 1990c; White and Svendsen 1990; Lacson and Morizot 1991; Cameron 1992; Cowie 1992). Cases in which gene frequencies are found to change over time allow the direct study of the causes of evolutionary change. However, assessing the genetic and morphological stability of populations is useful even when no change is detected because it has a direct bearing on the reliability of conclusions drawn from evolutionary studies that have sampled populations only once. It has been argued (e.g., Mickevich and Johnson 1976; Crother 1990) that gene frequencies should not be used in phylogenetic reconstruction because their putative lability through time makes them phylogenetically uninformative. If gene frequencies of populations are indeed subject to recurrent changes, this argument could be extended to any other inference drawn from genetic divergence determined at one

TABLE 1. Number of individuals sampled for every locus in each year from populations on the two coasts of Panama. All populations belong to *Excirolana braziliensis* except the population from Pta. Chame, which belongs to *Excirolana chamensis*. —, not sampled.

	Atlantic						Pacific											
	Bocas del Toro		Maria Chiquita		Shimmey Beach		Is. Adentro		Is. Culebra		Causeway Beach		Lab Beach		Is. Porcada		Pta. Chame	
Locus	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988
Alkph-1	32	27	30	37	23	83	24	41	47	42	37	28	23	48	20	27	31	28
Alkph-2	30	26	31	40	22	72	22	49	41	50	38	29	26	46	22	23	28	29
βGa	—	20	8	31	—	27	—	33	14	21	5	19	11	24	—	30	5	39
Fk	23	33	25	31	20	47	12	54	41	19	15	21	37	39	18	34	20	37
Fum	21	43	36	36	20	30	26	29	24	44	29	11	21	22	26	15	23	8
Got	16	49	35	28	25	50	20	28	28	30	30	21	31	29	20	22	23	44
M6pi	32	26	23	46	22	45	24	38	37	45	38	21	23	27	21	31	18	25
Mdh-1	20	33	25	30	21	66	27	42	45	25	32	19	51	52	39	25	26	29
Mdh-2	20	33	25	30	21	53	28	42	45	25	32	19	51	50	39	25	16	29
Peplt	19	22	20	33	20	43	28	24	23	22	36	21	34	39	31	24	20	20
Pgi	25	33	28	29	21	33	26	37	33	24	30	22	50	30	32	28	22	21
Pgm	20	27	30	25	21	26	6	32	35	23	18	21	36	20	13	12	19	24
Tpi	—	19	14	24	—	38	—	25	15	29	20	21	15	15	—	22	6	33

point in time, such as the importance of local adaptation *versus* gene flow, the degree of genetic structuring of populations through space, or any other reconstruction of genetic history. It is, therefore, important to assess the temporal variability of populations of any organism, but it is particularly interesting to study species with short generation times and high population subdivision because they have the greatest potential for rapid changes.

Excirolana braziliensis is a small (mean adult size 3.2 mm), marine isopod that lives in the intertidal area of sandy beaches. It is the numerically dominant species in many tropical sand beach communities of the New World (Dexter 1972, 1974, 1977, 1979; Glynn et al. 1975; Jaramillo 1978; Brusca and Iverson 1985). 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. Despite this wide range, its means of dispersal are limited. It has no larval stage. The female broods 4–17 embryos, then releases them into the parental habitat (Dexter 1977). Adults remain buried in the sand during low tide beach exposure. At high tide, they emerge into the water column to feed and may attach to prey, but they usually release after a few minutes (Brusca 1980). No reliable data exist on the generation time of this species, but Brusca and Iverson (1985) estimated the period of population turnover at about 4 mo.

Studies of the multivariate morphometrics of *E. braziliensis* from Panama (Weinberg and Starczak 1988) and from the entire range of the species (Weinberg and Starczak 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. Collections from 43 beaches from the entire range of *E. braziliensis* indicate that, with only two exceptions, each beach contains an overwhelming majority of one morph at any given time. Though the reasons for this pattern of nearly exclusive occupation of each beach by a single morph are unclear, intensive sampling of three beaches (Weinberg and Starczak 1988) revealed that it is not a sampling artifact. A comparison of allozymes and morphology of populations from 10 Panamanian beaches (Lessios and Weinberg 1994) found a nearly perfect correlation between the amount of morphological and genetic differentiation between populations, suggesting that morphological variation in space has a solid genetic underpinning. The same study revealed much genetic divergence between morphs and between populations of the same morph. Populations from each of 10 beaches studied have a unique combination of alleles, even though they may belong to the same morph and even though the physical distance separating them may be less than 1 km. F_{ST} statistics and private allele analysis indicate that gene flow between beaches is limited (Lessios and Weinberg 1993). Rates of migration calculated on the basis

of rare homozygotes of alleles otherwise absent from the resident population suggest that migrants between beaches exist, but they are rarely successful in transferring genes. In one area in which two genotypes coexist, there are significant heterozygote deficiencies (Lessios and Weinberg 1993). Thus, populations currently assigned to *Excirolana braziliensis* may be reproductively isolated from each other. Brusca and Weinberg (1987) described a new species, *E. chamensis,* from the Pacific coast of Panama. This species is included in the present study.

By examining samples of *E. braziliensis* from one locality, collected during a 17 yr period, Weinberg and Starczak (1988) concluded that the same morph can occupy a given beach for long periods of time. In this paper, we examine the genetic and morphological stability of nine populations of *E. braziliensis* on the two sides of the Isthmus of Panama, and of one population of *E. chamensis* from its type locality in the eastern Pacific. Our aim is to determine whether the apparent constancy suggested by morphological evidence from one beach holds true generally, and whether it is indicative of true genetic stability. For cases of genetic and morphological shifts through time, we attempt to determine the agents responsible for evolutionary change.

Materials and Methods

Excirolana braziliensis was sampled for electrophoretic and morphological analyses at three localities on the Atlantic and five localities on the Pacific coast of Panama. *Excirolana chamensis* was collected at Punta Chame, in the Bay of Panama (tables 1, 2; fig. 1 in Lessios and Weinberg 1994). Duplicate samples were obtained from each beach, one between February 4 and February 26, 1986, and a second between September 17 and October 31, 1988. For morphological measurements, we also used an earlier sample from each beach, collected between June 23, 1984 and March 4, 1985. In one locality, Isla Porcada, two morphological samples, one collected on December 19, 1984, the other on May 8, 1985 were pooled to provide an adequate sample size (table 2). *Excirolana braziliensis* was also sampled in 1986 and 1988 at one additional locality (Perico) for morphometric analysis. All three morphs identified by Weinberg and Starczak (1988, 1989) are represented in the samples (table 2). Isopods were collected by sieving (0.5 mm hole size) the uppermost 5-cm layer of sand from the high intertidal zone, transported to the laboratory in plastic bags full of moist sand, and frozen in liquid nitrogen for electrophoresis, or preserved in 95% ethanol for morphometric analysis.

TABLE 2. Number of individuals of each sex measured from each locality in each year for morphometric analysis. *Excirolana braziliensis* was sampled in all localities except Punta Chame. Morph to which residents of each beach belong according to Weinberg and Starczak (1988, 1989) is shown in parentheses under the locality name: **C**, Caribbean morph; **C′**, Caribbean-like morph in the Pacific; **P**, Pacific morph. Not sampled is indicated by —. Isla Culebra is identified as "Boy Scout beach" in Weinberg and Starczak (1988, 1989). Samples from 1984 and 1985 of Isla Porcada were pooled for statistical analyses.

Locality	Year	♂	♀
Bocas del Toro	1984	7	11
(C)	1986	6	8
	1988	8	8
Maria Chiquita	1984	11	11
(C)	1986	8	8
	1988	8	8
Shimmey Beach	1985	11	12
(C)	1986	6	6
	1988	8	9
Is. Adentro	1985	9	11
(C′)	1986	—	—
	1988	8	9
Is. Culebra	1985	12	10
(C′)	1986	7	5
	1988	10	8
Causeway Beach	1985	8	11
(P)	1986	6	4
	1988	—	—
Lab Beach	1985	10	15
(P)	1986	6	6
	1988	9	9
Is. Porcada	1984	5	8
(P)	1985	2	4
	1986	8	6
	1988	10	10
Perico	1984	—	—
(P)	1986	6	7
	1988	7	9
Punta Chame	1984	9	11
E. chamensis	1986	5	5
	1988	—	—

Electrophoresis.—Of 14 enzymes used to assess spatial variation (Lessios and Weinberg 1994), the following 11 gave scorable results in both years (presumptive locus designation in parentheses): Alkaline phosphatase (*Alkph-1* and *Alkph-2*), N-Acetyl-β-glucosaminase (*βGa*), Fructokinase (*Fk*), Fumarate dehydrogenase (*Fum*), Glutamate-oxaloacetate transaminase (*Got*), 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 13 scorable loci. Assaying methods are presented in Lessios and Weinberg (1994). 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 Results), this standardization was adequate, though homogenate from the same individual could not (because of the small amount available) be run in multiple gels.

Analysis of Genetic Data.—Gene frequencies of each polymorphic locus at each locality were first compared between years with simple χ^2 tests to determine whether sampling error alone could explain observed differences. Significance levels were adjusted for the number of tests performed with the standard Bonferroni inequality (Rice 1989). Cases in which χ^2 tests indicated significant differences in allele frequency between years were subjected to the analysis suggested by Waples (1989a). This method estimates the probability that differences between gene frequencies of the same population, sampled in different generations, are caused by nothing more than sampling error and genetic drift. Unlike the standard χ^2 test, it takes into account the increased variance in frequencies that genetic drift will cause between generations. Given sample sizes, values for effective population size, and total population size, it yields significant test statistics when stochastic factors alone cannot account for the shifts of gene frequencies from generation to generation. Program TEMPTEST (Waples 1989a) was used to carry out this 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, length of uropodal exopod) were measured on an approximately equal number of males and females (table 2). A ninth character, length of appendix masculinum, which is only present in males, was also measured. Methods of measurement and a discussion of the characters are given in Weinberg and Starczak (1988, 1989). Spatial morphological variation is discussed in Lessios and Weinberg (1994).

Analysis of Morphometric Data.—Two separate analyses were conducted, one with data from both sexes combined and involving the eight characters of gross morphology and another limited to data from males and including the length of appendix masculinum. 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 (55.37%–56.82% in the present case). We used the scores of the first principal component, after removal of two outliers (see below), to analyze changes in body shape through time in each locality. The first principal component represents shape, because the size variation is removed by the Burnaby adjustment. It was found adequate for analysis of morphological variation through space by Weinberg and Starczak (1989) and appears to perform well in the analysis of temporal variation as well. A parallel analysis using the first five principal components did not result in substantially different conclusions from the analysis limited to only the first one. To test whether inhabitants of each beach changed in morphology over time, we performed two- and three-way ANOVAs with locality, year of collection, and gender (when eight variables from both sexes were used) as the independent variables, and the first principal component as the response variable. The independent variables were analyzed as fixed factors. Because of the unbalanced design resulting from missing data from some localities in a particular year, comparisons among the three years were performed in pairwise fashion. We used Tukey's Honest Significant Difference test for unequal sample sizes to determine which collections from the same locality differed from each other.

Results

Isozymes

The genetic constitution of most populations remained constant from 1986 to 1988. Shifts in gene frequencies were slight; simple χ^2 tests indicate that differences between years are not significantly different in all loci of all populations, except those from Isla Porcada (table 3). Two loci, *Mdh-1* and *Pgm*, in this population changed significantly from 1986 to 1988. Allele $Mdh\text{-}1^a$ was completely replaced by allele $Mdh\text{-}1^b$, which in 1986 was represented only in a single homo-

zygote. To determine whether changes in these two loci were caused by the combined effects of genetic drift and sampling error, we needed to provide Waples's (1989a) model with possible values for total population size and number of elapsed generations. We used the number of individuals we collected at Isla Porcada during 1988 (85 individuals, collected for analysis in all loci, plus morphometrics) as an estimate of minimum possible total population size. To estimate the bounds of the number of possible generations that were produced between the two samples, we used our observations that in seawater tables *Excirolana braziliensis,* living in sand and simulated tides, took a minimum of 3 mo to reach sexual maturity and Zuniga et al.'s (1985) data, indicating that in a natural population in Chile with essentially nonoverlapping generations, a maximum of 10 mo elapsed between peak juvenile recruitment and peak percent of ovigerous females. Thus, 2–11 generations is a range of possible estimates of the number of generations that could have been produced in the 31 mo between samples. We considered it prudent, however, to evaluate the effects of a reduction of time to maturation by an additional month, which can bring the number of generations to 14. For this range of generations, we calculated Waples's statistics for different values of effective population size, N_e, and asked how low N_e had to become before a nonsignificant comparison of gene frequencies could be obtained. Dexter (1977) reports that in *E. braziliensis* at Panama, sex can be differentiated at a size of 2 mm, that females become ovigerous at 2.9 mm, and they most likely produce only one brood through their lifetime. Thus, the individuals we used for our genetic analysis, being longer than 3 mm, consisted of adults that may or may not have reproduced. Accordingly, we calculated Waples's (1989a) statistics for both his sampling Plan I (sampling after reproduction), and his sampling Plan II (sampling before reproduction). As table 4 indicates, certain combinations of very low effective population size, low total population size, and many generations could explain the frequency shifts in *Pgm* as the result of drift and sampling error. However, no such combination yields anything but a highly significant χ^2 value for the changes in *Mdh-1,* suggesting that some factor other than genetic drift and sampling error has caused gene frequency changes at this locus. N_e values in table 4 should be considered as the harmonic means of the actual effective population sizes over the number of elapsed generations, which makes it all the more improbable that genetic drift would account for the changes documented here.

In both *Mdh-1* and *Pgm,* the alleles that increased in frequency in *E. braziliensis* at Isla Porcada are those that predominate in *E. chamensis* at Punta Chame. It might, therefore, be thought that gene flow from *E. chamensis* was responsible for the significant shifts at these loci. However, this interpretation is not consistent with the results from other loci. *Alkph-2, βGa,* and *Tpi* of the 1988 Isla Porcada sample were fixed for alleles that differed from those of *E. chamensis* (table 3). An influx of genes from Isla Adentro, which is located nearby, is also not a viable possibility, because there are no shared alleles between this population and Isla Porcada in *Alkph-2, Got,* and *M6pi* (table 3).

Morphology

Figure 1 shows the first principal-component scores of each individual from each locality in each year. Two outliers are obvious: one individual in 1986 at Isla Culebra, instead of displaying the "Caribbean-like" morphology typical of this beach (Weinberg and Starczak 1988, 1989), belongs to the "Pacific" morph; another individual at Isla Porcada in 1984 does not belong to the Pacific-morph characteristic of this beach but instead resembles the Caribbean-like morph. Such rare representatives of another population have been found in other localities sampled for morphology (Weinberg and Starczak 1989, their fig. 3). They are also evident in our electrophoretic analysis as homozygotes of an allele that is otherwise absent from the resident population (Lessios and Weinberg 1993). We believe that these individuals represent recent migrants from another beach (see also Weinberg and Starczak 1988; Lessios and Weinberg 1993). Though such outliers are important in providing an estimate of the number of isopods that migrate between beaches and in understanding the cause of genetic changes at Isla Porcada, their inclusion would violate the distributional requirements of the statistical comparisons between years. These two outliers, therefore, were excluded from our ANOVA analyses. At Isla Porcada, in which it was necessary to pool 1984 and 1985 samples, a comparison of principal-component mean scores of the 2 yr showed no significant difference (two-sample t-test: $t = 0.589$, $P = 0.580$).

TABLE 3. Allele frequencies in each locus, year, and locality, and significance of χ^2 tests comparing samples from the two years. Alleles are listed in order of increasing mobility away from the origin. χ^2 tests were not performed in loci that were monomorphic for the same allele in both years. Significance values were adjusted for the 50 tests performed, using the Bonferroni inequality. NS, not significant. No data indicated by —. See table 1 for sample sizes for each locus.

		Atlantic						Pacific											
		Bocas del Toro		Maria Chiquita		Shimmey Beach		Is. Adentro		Is. Culebra		Causeway Beach		Lab Beach		Is. Porcada		Pta.Chame	
Locus	Allele	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988
				χ^2 NS												χ^2 NS		χ^2 NS	
Alkph-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
	b	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.975	0.963	1.000	0.982
	c	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.018
		χ^2 NS						χ^2 NS		χ^2 NS								χ^2 NS	
Alkph-2	a	0.000	0.038	0.000	0.000	0.000	0.000	1.000	0.939	1.000	0.960	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.966
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.034
	c	1.000	0.962	1.000	1.000	1.000	1.000	0.000	0.061	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
βGa	a	—	0.000	0.000	0.000	—	0.000	—	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	0.000	1.000	1.000
	b	—	1.000	1.000	1.000	—	1.000	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	1.000	0.000	0.000
	c	—	0.000	0.000	0.000	—	0.000	—	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	0.000	0.000	0.000
	d	—	0.000	0.000	0.000	—	0.000	—	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	0.000	0.000	0.000
						χ^2 NS		χ^2 NS		χ^2 NS								χ^2 NS	
Fk	a	0.000	0.000	0.000	0.000	0.100	0.000	0.083	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
	b	1.000	1.000	1.000	1.000	0.900	1.000	0.917	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000
				χ^2 NS		χ^2 NS						χ^2 NS				χ^2 NS			
Fum	a	0.000	0.000	0.014	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.058	0.000	0.000	0.000
	b	1.000	1.000	0.986	1.000	0.950	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	1.000	0.942	1.000	1.000	1.000
										χ^2 NS						χ^2 NS			
Got	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	1.000	1.000	1.000	1.000	1.000	0.977	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
						χ^2 NS		χ^2 NS										χ^2 NS	
M6pi	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.960
	b	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	1.000	1.000	1.000	1.000	1.000	0.989	1.000	0.987	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040

TABLE 3. Continued.

		Atlantic						Pacific											
		Bocas del Toro		Maria Chiquita		Shimmey Beach		Is. Adentro		Is. Culebra		Causeway Beach		Lab Beach		Is. Porcada		Pta.Chame	
Locus	Allele	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988	1986	1988
						χ^2 NS						χ^2 NS				χ^2 ***		χ^2 NS	
Mdh-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.947	1.000	1.000	0.974	0.000	0.000	0.000
	b	1.000	1.000	1.000	1.000	0.976	1.000	1.000	1.000	1.000	1.000	0.000	0.053	0.000	0.000	0.026	1.000	0.981	1.000
	c	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
				χ^2 NS		χ^2 NS													
Mdh-2	a	0.000	0.000	0.020	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	0.980	1.000	0.976	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS	
Peplt	a	0.000	0.000	0.025	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.079	0.000	0.175	0.045	0.075	0.116	0.089	0.167	0.109	0.023	0.000	0.000	0.044	0.000	0.000	0.021	0.075	0.025
	c	0.895	1.000	0.750	0.924	0.925	0.837	0.786	0.813	0.761	0.955	0.972	0.952	0.926	0.962	0.935	0.854	0.925	0.875
	d	0.026	0.000	0.025	0.000	0.000	0.023	0.125	0.021	0.130	0.023	0.028	0.048	0.029	0.026	0.065	0.125	0.000	0.100
	e	0.000	0.000	0.025	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS		χ^2 NS			
Pgi	a	0.020	0.000	0.018	0.000	0.024	0.000	0.000	0.000	0.000	0.000	1.000	0.955	1.000	0.967	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.078	0.000	0.000	0.000
	c	0.920	1.000	0.982	1.000	0.952	1.000	0.942	1.000	0.848	1.000	0.000	0.045	0.000	0.033	0.891	1.000	1.000	1.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	e	0.040	0.000	0.000	0.000	0.024	0.000	0.058	0.000	0.121	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000
	f	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		χ^2 NS		χ^2 NS				χ^2 NS				χ^2 NS				χ^2 *		χ^2 NS	
Pgm	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083
	b	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.542	0.947	0.917
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	1.000	0.981	0.950	1.000	1.000	1.000	0.750	0.984	1.000	1.000	1.000	0.976	1.000	1.000	0.923	0.458	0.053	0.000
	e	0.000	0.019	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000
																		χ^2 NS	
Tpi	a	—	1.000	1.000	1.000	—	0.987	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	0.000	1.000	0.985
	b	—	0.000	0.000	0.000	—	0.013	—	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	0.000	0.000	0.015
	c	—	0.000	0.000	0.000	—	0.000	—	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	1.000	0.000	0.000

* $P < 0.05$; *** $P < 0.001$.

TABLE 4. Use of Waples's (1989a) method to test whether changes in gene frequencies from 1986 to 1988 at Isla Porcada are caused by sampling error and genetic drift alone. Statistics were evaluated for the two loci that gave significant results in simple χ^2 tests (see table 3). Plan I, sampling after reproduction; Plan II, sampling before reproduction. The hypothesis that stochastic factors account for the changes cannot be rejected if there is a possible combination of number of elapsed generations, effective population size (N_e), and total population size (N) that could have produced the gene frequency changes seen on table 3. For every possible number of elapsed generations, the table lists the highest value of N_e that retains nonsignificance in χ^2. In generation numbers at which a second, larger value of N permits an increase in N_e without producing a significant χ^2 value, the smallest N value that allows this transition is also given. In cases in which no combination of generation number, N_e, and N can produce a nonsignificant χ^2, the value and significance of the lowest possible χ^2 is given. In sampling Plan II, N is infinitely large. For each value of N_e under Plan I, values of χ^2 decrease as N increases, but they can never become less than those of Plan II. Thus, the position of the χ^2 value for Plan II in the table indicates the highest N_e, which can give a nonsignificant χ^2 value under Plan I, no matter how much N might be increased.

No. of generations	*Pgm*				*Mdh-1*			
	Plan I			Plan II	Plan I			Plan II
	N_e	N	χ^2	χ^2	N_e	N	χ^2	χ^2
2	2	85	4.60*	4.33*	2	85	53.61***	40.91***
3	2	85	3.81	3.62	2	85	44.72***	35.45***
4	2	85	3.38	3.23	2	85	39.79***	32.25***
5	3	85	3.72	3.54	2	85	36.75***	30.21***
6	3	85	3.45		2	85	34.77***	28.85***
6	4	160	3.84	3.74				
7	4	85	3.68	3.50	2	85	33.41***	27.90***
8	4	85	3.48		2	85	32.46***	27.23***
8	5	90	3.84	3.66				
9	5	85	3.66		2	85	31.78***	26.75***
9	6	250	3.84	3.78				
10	6	85	3.80	3.61	2	85	31.29***	26.40***
11	6	85	3.65		2	85	30.94***	26.15***
11	7	130	3.84	3.71				
12	7	85	3.76		2	85	30.67***	25.96***
12	8	340	3.84	3.79				
13	7	85	3.64		2	85	30.48***	25.82***
13	8	100	3.83	3.67				
14	8	85	3.74		2	85	30.34***	25.72***
14	9	170	3.84	3.74				

* $0.01 < P < 0.05$; *** $P < 0.001$.

Which of the populations underwent statistically significant changes in morphology over time? Our two-way ANOVAs of the first principal component comprising all characters in males (table 5) and our three-way ANOVAs of the first principal component including nonreproductive characters in both sexes (table 6) show no significant main effects of time alone, indicating that in most localities morphology remained stable. However, interaction terms of time with locality are significant in ANOVAs comparing 1984–1985 to 1988, and 1986 to 1988, but not significant in the ANOVA comparing 1984–1985 to 1986 (tables 5, 6). Thus, the ANOVA analyses indicate that though populations remained morphologically stable at all localities from 1984 to 1986, and at most localities from 1986 to 1988, one or more of these populations changed from 1986 to 1988. A posteriori multiple range tests (table 7) show that Isla Porcada is the only locality at which morphology changed significantly.

DISCUSSION

When allozyme frequencies changed at Isla Porcada between 1986 and 1988, so did the morphology of the population. This correlation between genetic constitution as represented by allozymes on the one hand, and morphology as represented by the first principal component on the other, holds true not only in variation through time, documented here, but also in variation through space (Lessios and Weinberg 1994). It is, therefore, very probable that the morphological stability seen during 3 to 4 yr in all localities

TABLE 5. Results of three two-way ANOVAs testing whether scores of the first principal component differ between pairs of years. Data are from the measurements of males only. Measurements of the appendix masculinum are included in the calculation of the first principal-component scores. Probabilities corresponding to *F*-values calculated for each main effect and interaction term are shown.

Comparison	Effects		
	Locality	Time	Locality × time
1984–1985 vs 1986	< 0.001	0.810	0.130
1984–1985 vs 1988	< 0.001	0.368	< 0.001
1986 vs 1988	< 0.001	0.454	< 0.001

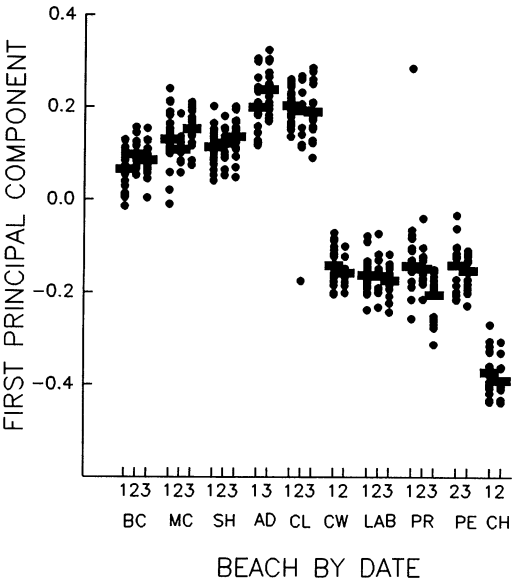

FIG. 1. First principal component scores of each individual of *Excirolana* at every beach in every year. Rectangles indicate the mean of each sample. BC, Bocas del Toro; MC, Maria Chiquita; SH, Shimmey Beach; AD, Isla Adentro; CL, Isla Culebra (Boy Scout); CW, Causeway Beach; LAB, Lab Beach; PR, Isla Porcada; PE, Isla Perico; CH, Punta Chame. 1, 1984–1985; 2, 1986; 3, 1988. The means do not include outliers in the 1986 sample of Isla Culebra and in the 1984–1985 sample of Isla Porcada.

but one, and during 17 yr in Isla Culebra (Weinberg and Starczak 1988) reflects genetic stability. Thus, the main conclusion of this study is that genetic constitution of local populations of *Excirolana braziliensis* and *E. chamensis* tends to stay constant through time, despite the population subdivision imposed by habitat specialization and developmental mode (Lessios and Weinberg 1994), and despite the quick turnover of the populations because of short generation time. Occasionally, however, a population resident in a locality may change significantly both in morphology and isozymes as seen at Isla Porcada. We examined either morphology or isozymes in five populations for 4 yr, in four populations for 3 yr, and in two populations for 2 yr, which leads to the conclusion that in *Excirolana* such changes would be expected to occur in 1 of 36 population years or that the probability that genetic change will occur in a given locality in a given year is 0.028. If we assume that this species has three generations per year, this translates to a probability of 0.009 per generation.

Other studies involving assessments of genetic stability through time are divided between those that found that genetic constitution of populations tends to stay stable (e.g., Bishop 1969; Berger 1971; Redfield 1973; Koehn and Williams 1978; Powers and Place 1978; Bulnheim and Scholl 1981; Burton and Feldman 1981; Cavener and Clegg 1981; Mihok et al. 1983; Lessios 1985; McClenaghan et al. 1985; Daly and Patton 1990; Loxdale and Brookes 1990; Waples 1990c; White and Svendsen 1990; Cowie 1992) and those that have found substantial change during relatively

TABLE 6. Results of three three-way ANOVAs testing for differences in scores of the first principal component between pairs of years. Measurements from both female and male specimens are included. Measurements of the appendix masculinum are excluded. Probabilities corresponding to *F*-values calculated for each main effect and interaction term are shown.

Comparison	Effects						
	Locality	Time	Gender	Locality × time	Locality × gender	Gender × time	Locality × gender × time
1984–1985 vs 1986	< 0.001	0.636	0.313	0.228	0.040	0.590	0.298
1984–1985 vs 1988	< 0.001	0.973	0.225	< 0.001	0.090	0.319	0.024
1986 vs 1988	< 0.001	0.194	0.078	< 0.001	0.004	0.615	0.032

TABLE 7. Results of Tukey's HSD multiple comparison tests, performed to determine which years within localities compared in tables 5 and 6 are significant. Lines on the same level join years in which the morphological variables did not change significantly at the $P = 0.05$ level. Results are the same whether eight variables from both sexes are used, or the appendix masculinum is added and data from males alone are used. ND, no data.

	1984–1985	1986	1988
Bocas del Toro	———		
Maria Chiquita	———		
Shimmey Beach	———		
Is. Adentro	———	ND	———
Is. Culebra	———		
Causeway Beach	———		ND
Lab Beach	———		
Is. Porcada	———	———	———
Perico	ND	———	
Pta. Chame	———		ND

short periods of time (e.g., Dobzhansky 1943; Tamarin and Krebs 1969; Gaines and Krebs 1971; Krimbas and Tsakas 1971; Dobzhansky and Ayala 1973; Gaines et al. 1978; Steiner 1979; Smith and Patton 1984). Given that investigators are more prone to study species and loci in which temporal genetic changes are suspected, (all studies that have found temporal instability involve *Drosophila* or small mammals, and only Steiner's involves more than six loci), and that short-term genetic stability is unlikely to be reported (we would have not written an article about temporal stability in *Excirolana* had we not observed the sudden change at Isla Porcada), we believe that the data base as a whole supports the view that gene frequencies in populations tend to stay sufficiently stable over time to allow reconstructions of their genetic histories based on the assumption that they are close to equilibrium. Though there will undoubtedly be cases in which a single sample of a population through time produces misleading results, by and large there is no reason to be more suspicious of phylogenetic reconstructions based on gene frequencies (e.g., Crother 1990) than of those based on any other character. Indeed, analyses of morphology and gene frequencies in *Excirolana* produce the same conclusions and would lead to the same mistakes.

The general result that populations tend to stay genetically stable for a few years is comforting with regards to the confidence one can have in conclusions reached by studies that have taken only one genetic sample through time, but—from the point of view of documenting and understanding evolution—relatively uninteresting. It is the exception in our data that has the potential of revealing processes that are responsible for the present-day genetic structure of *Excirolana*. We, therefore, devote the remainder of the discussion to what may have happened between 1986 and 1988 at Isla Porcada to precipitate the drastic shift in allele frequencies of two loci and in overall morphology, and to what this means for the mode of evolution of *E. braziliensis*. Mutation pressure can be ignored as a possible cause of genetic change during the short time period considered here; thus, we need to consider four other possible explanations: (1) sampling accidents, (2) genetic drift, (3) directional selection, and (4) immigration from another population.

The results from Isla Porcada might have been caused by a sampling accident if two noninterbreeding populations always existed there, and we accidentally collected significantly different proportions of each in different years. This, however, is unlikely for the following reasons. (1) The sand samples sieved for isopods were taken by the same person (J.R.W.) at low tide in multiple sites of the beach in all 4 yr. If the two hypothetical populations overlapped spatially, they would have both appeared in all three samples; if they were segregated, the same population would have been sampled in all 3 yr. (2) Extensive sampling of three other beaches by Weinberg and Starczak (1988) had found that only one morph was present at a given time throughout each beach. (3) Forty one out of 43 beaches sampled by Weinberg and Starczak (1989) contained only one morph. (4) In Santelmo, one of the two beaches where two morphs are known to coexist, samples were taken in 1984 and in 1986. On both occasions, the two morphs were detected in proportions that were roughly equal between years. Thus, coexistence of two morphs is rare, and when it is present, our sampling regime can detect it regardless of time of collection. Thus, we doubt that a sampling accident could explain the change in Isla Porcada.

Waples's test indicated that genetic drift may explain changes seen in *E. braziliensis* in Isla Porcada in *Pgm* but not in *Mdh-1*. The change in gene frequencies seen in the latter locus were too great to be explained by any possible value of effective population size or number of elapsed generations. Even for *Pgm*, genetic drift is impossible for any harmonic mean of N_e of more

than nine individuals, taken for 14 generations. It is also unlikely that genetic drift could cause, during this short time, the significant shifts in multivariate morphology. For these reasons, we doubt that changes in Isla Porcada could have arisen by drift, even though we believe that small population size has been important in the evolution of genetic patterns of *Excirolana* (see below).

Another possible explanation for the observed changes is that extremely strong selection caused the increase in frequency of a genotype that was rare in 1984 and 1986. Concordant with this hypothesis is the observation of a shift in two loci but not in others. Lewontin and Krakauer (1973) have stressed that selection can be differentiated from genetic drift and gene flow by the fact that it alone can affect each locus differently. Although many statistical tests exist for the detection of the effects of natural selection from temporally spaced samples, (e.g., Fisher and Ford 1947; Lewontin and Krakauer 1973; Templeton 1974; Schaffer et al. 1977; Wilson 1980; Watterson 1982), they are all applicable only to closed populations. No way exists to distinguish statistically the effects of selection from those of immigration from a population with unknown genetic constitution. With our present data, we cannot completely exclude the possibility that the dominant genotype at Isla Porcada selectively died after 1986. If morphology and isozymes at the *Mdh-1* and *Pgm* locus are under linkage disequilibrium because they both characterize genotypes unable to mate with each other, then natural selection is not such an unreasonable hypothesis despite the short time and the large differences between samples. However, this hypothesis requires that the genotypes were differentially affected by a cause of mortality present between 1986 and 1988 but absent before 1986.

Immigration from another locality is the only remaining possibility as an agent of change after 1986 at Isla Porcada, and the one we favor as the most likely explanation. Rare genotypes in *Mdh-1* and *Pgm* were present in 1986 as single homozygotes of the rare allele at each of these loci. These were probably migrants from an unsampled source population that was later to replace the Isla Porcada population. Lack of any heterozygotes between rare and common alleles in either locus during 1986 suggests that such migrants, perhaps because of their recent arrival and rarity, perhaps because of active discrimination, or perhaps because of genomic incompatibility, did not produce hybrids with the resident population. Thus, migration in this case is not synonymous with gene flow. We postulate that at some point between 1986 and 1988 the resident population was replaced by the migrants. How this happened is impossible to tell without ecological data. It is possible that a change in oceanographic conditions increased the rate of immigration, which in turn caused greatly increased frequencies of the previously rare genotype and a corresponding overall increase in the population of *E. braziliensis*. We doubt that this happened, because isopod density at Isla Porcada was low in 1988, and because we would have expected our 1988 sample to include at least a few individuals with allele $Mdh\text{-}1^a$ if they were present. It is also possible that increased immigration, reproduction, or survival rate of the previously rare genotype brought about the extinction of the previous dominant through competitive exclusion, without an increase in the overall population density. Another possibility is that the previously dominant genotype suffered selective mortality (see above). Although we have no data to help us select between these possibilities, we believe that the most parsimonious explanation is also the most likely: The resident population of Isla Porcada was severely reduced or became extinct because of extreme conditions at the upper intertidal, such as sustained rains at low tide. The species can tolerate fresh water for only 4 h (Dexter 1977), and Isla Porcada is located at the mouth of Rio Santa Lucia. If the immigration rate of the rare genotype remained constant after this event, the net result would be a large increase in the frequency of the genotype that previously was represented in our samples by only a few individuals (and a reduction in population density). Thus, we conclude that at Isla Porcada after 1986, there was either selective survival of one genotype or replacement of the resident population by another.

If we are correct in thinking that the population that existed at Isla Porcada until 1986 became extinct and that the area was colonized by a new genotype from another locality, then on the metapopulation (Levins 1970; Olivieri et al. 1990) level evolution did not take place. However, such local extinctions and recolonizations may explain the high genetic differentiation found between local demes of the same morph of *E. braziliensis* (Lessios and Weinberg 1994). When extinction and recolonization occur frequently,

and the source of colonizers consists of a mixture of several populations, they are believed to homogenize the species gene pool (Maruyama and Kimura 1980; Slatkin 1985; Ewens et al. 1987; Barton 1988; Lande 1992). However when the recolonizing propagules are drawn from a single population, and when their number is low, they exert a greatly differentiating effect caused by genetic drift (Wright 1940; Selander 1975; Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990; McCauley 1991; Lande 1992). Though we lack the necessary information on colonization and migration rates to determine exactly where *Excirolana* should be placed with respect to the models predicting differentiation or homogenization, we believe that extinction and recolonization in this genus should over the years enhance differentiation for the following reasons. (1) In Isla Porcada, both morphometrics and allozymes indicate the arrival from 1986 to 1988 of a single new genotype. No evidence exists of multiple genotypes that could have been immigrants from additional localities. Thus, *Excirolana* is more likely to fit in Slatkin's (1977) and Wade and McCauley's (1988) "propagule pool" model (all colonists derived from a single population) than the "migrant pool" model (colonists originate from several populations); the propagule pool model is much more likely to result in population differentiation (Wade and McCauley 1988). (2) Immigrants of *E. braziliensis* with distinct genotypes appear not to mate randomly and produce hybrids with members of the local population. Thus, gene flow between existing populations is low (Lessios and Weinberg 1993). The brooding reproductive mode and an estimated average gestation period of 37 d (Dexter 1977) make this species a good potential colonizer of empty beaches, even if dispersing individuals do not move far. As Wade and McCauley (1988) have pointed out, species that store sperm or bear live young but move little can have a high average colonization rate and a low gene-flow rate, a condition that promotes differentiation. Thus, extinction and recolonization likely would result in interpopulation divergence in *Excirolana*.

Whether the extremely rapid change seen in isozymes and morphology at Isla Porcada is caused by extinction and colonization, selection, or any other cause, cannot be settled except by ecological data. However, it is probable that infrequent but drastic temporal changes, involving passage through small population size, are responsible over the history of the species for the large amounts of divergence we have seen between local populations of *Excirolana* both on the Atlantic and on the Pacific coasts of Panama.

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Literature Cited

Barton, N. H. 1988. Speciation. Pp. 185–218 *in* A. A. Myers and P. S. Giller, eds. Analytical biogeography. Chapman and Hall, London.

Berger, E. M. 1971. A temporal survey of allelic variation in natural and laboratory populations of *Drosophila melanogaster*. Genetics 67:121–136.

Bishop, J. A. 1969. Changes in genetic constitution of a population of *Sphaeroma rugicauda* (Crustacea: Isopoda). Evolution 23:589–601.

Blouw, D. M., S. D. Saxon, and E. M. P. Chadwick. 1988. Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock, and implications for stock identification. Canadian Journal of Fisheries and Aquatic Sciences 45:1330–1339.

Brusca, R. C. 1980. Common intertidal invertebrates of the Gulf of California, 2d ed. University of Arizona Press, Tucson.

Brusca, R. C., and E. W. Iverson. 1985. A guide to the marine isopod Crustacea of Pacific Costa Rica. Revista de Biologia Tropical 33:1–77.

Brusca, R. C., and J. R. Weinberg. 1987. A new isopod crustacean from Pacific Panama, *Excirolana chamensis* new species (Isopoda: Flabellifera: Cirolanidae). Contributions in Science Los Angeles County Museum 392:11–17.

Bulnheim, H. P., and A. Scholl. 1981. Genetic variation between geographic populations of the amphipods *Gammarus zaddachi* and *G. salinus*. Marine Biology 64:105–115.

Burnaby, T. P. 1966. Growth-invariant discriminant functions and generalized distances. Biometrics 22: 96–110.

Burton, R. S., and M. W. Feldman. 1981. Population genetics of *Tigriopus californicus*. II. Differentiation among neighboring populations. Evolution 35: 1192–1205.

Cameron, R. A. D. 1992. Change and stability in *Cypaea* populations over 25 years: a case of climatic selection. Proceedings of the Royal Society of London B 248:181–187.

Cavener, D. R., and M. T. Clegg. 1981. Temporal stability of allozyme frequencies in a natural population of *Drosophila melanogaster*. Genetics 98: 613–623.

Cowie, R. H. 1992. Shell pattern polymorphism in a 13-year study of the land snail *Theba pisana* (Muller) (Pulmonata: Helicidae). Malacologia 34:87–97.

Crother, B. I. 1990. Is some better than none or do allele frequencies contain phylogenetically useful information? Cladistics 6:277–281.

Daly, J. C., and J. L. Patton. 1990. Dispersal, gene glow, and allelic diversity between local populations of *Thomomys bottae* pocket gophers in the coastal ranges of California. Evolution 44:1283–1294.

DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston. 1987. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. Genetics 116:215–223.

Dexter, D. M. 1972. Comparison of community structure in a Pacific and an Atlantic Panamanian sandy beach. Bulletin of Marine Science 22:449–462.

———. 1974. The sandy beach fauna of the Pacific and Atlantic coasts of Costa Rica and Colombia. Revista de Biologia Tropical 22:51–66.

———. 1977. Natural history of the Pan-American sand beach isopod *Excirolana braziliensis* (Crustacea: Malacostraca). Journal of Zoology (London) 183:103–109.

———. 1979. Community structure and seasonal variation in intertidal Panamanian sandy beaches. Estuarine and Coastal Marine Sciences 9:543–558.

Dobzhansky, T. 1943. Genetics of natural populations. IX. Temporal changes in the composition of populations of *Drosophila pseudoobscura*. Genetics 28:162–186.

———. 1947. A directional change in the genetic constitution of a natural population of *Drosophila pseudoobscura*. Heredity 1:53–64.

Dobzhansky, T., and F. J. Ayala. 1973. Temporal frequency changes of enzyme and chromosomal polymorphisms in natural populations of *Drosophila*. Proceedings of the National Academy of Sciences, USA 70:680–683.

Ehrlich, P., and L. G. Mason. 1966. The population biology of the butterfly *Euphydryas editha*. III. Selection and the phenetics of the Jasper Ridge colony. Evolution 20:165–173.

Ewens, W. J., P. J. Brockwell, J. M. Gani, and S. I. Resnick. 1987. Minimum viable population size in the presence of catastrophes. Pp. 59–68 *in* M. E. Soulé, ed. Viable populations for conservation. Cambridge University Press, Cambridge.

Fisher, R. A., and E. B. Ford. 1947. The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. Heredity 1:143–174.

Gaines, M. S., and C. J. Krebs. 1971. Genetic changes in fluctuating vole populations. Evolution 25:702–723.

Gaines, M., L. R. McClenaghan, and R. K. Rose. 1978. Temporal patterns of allozymic variation in fluctuating populations of *Microtus ochrogaster*. Evolution 32:723–739.

Glynn, P. W., D. M. Dexter, and T. E. Bowman. 1975. *Excirolana braziliensis* a Pan-American sand beach isopod: taxonomic status, zonation, and distribution. Journal of Zoology (London) 175:509–521.

Gyllensten, U. 1985. Temporal allozyme frequency changes in density fluctuating populations of willow grouse (*Lagopus* L.). Evolution 39:115–121.

Jaramillo, E. 1978. Zonación y estructura de la comunidad macrofaunistica en playas de arena del Sur de Chile (Mehuin, Valdivia). Studies on the Neotropical Fauna and Environment 17:71–92.

Koehn, R. K., and G. C. Williams. 1978. Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. II. Temporal stability of geographic patterns. Evolution 32:624–637.

Kohn, P. H., and R. H. Tamarin. 1978. Selection at electrophoretic loci for reproductive parameters in island and mainland voles. Evolution 32:15–28.

Krimbas, C. B., and S. Tsakas. 1971. The genetics of *Dacus oleae* V. Changes of esterases polymorphism in a natural population following insecticide control. Selection or drift? Evolution 25:454–460.

Lacson, J. M., and D. C. Morizot. 1991. Temporal genetic variation in subpopulations of bicolor damselfish (*Stegastes partitus*) inhabiting coral reefs in the Florida Keys. Marine Biology 110:353–357.

Lande, R. 1992. Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. Evolution 46:381–389.

Lessios, H. A. 1985. Genetic consequences of mass mortality in the Caribbean sea urchin *Diadema antillarum*. Proceedings of the Fifth Coral Reef Congress 4:119–126.

Lessios, H. A., and J. R. Weinberg. 1993. Migration, gene flow, and reproductive isolation between and within morphotypes of the isopod *Excirolana* in two oceans. Heredity 71:561–573.

———. 1994. Genetic and morphological divergence among morphotypes of the isopod *Excirolana* on the two sides of the Isthmus of Panama. Evolution 48:530–548.

Levins, R. 1970. Extinction. Lectures on Mathematics in the Life Sciences 2:75–107.

Lewontin, R. C., and J. Krakauer. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics 74: 175–195.

Loxdale, H. D., and C. P. Brookes. 1990. Temporal genetic stability within and restricted migration (gene flow) between local populations of the blackberry-grain aphid *Sitobion fragariae* in South-East England. Journal of Animal Ecology 59:497–514.

Maruyama, T., and M. Kimura. 1980. Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. Proceedings of the National Academy of Sciences, USA 77:6710–6714.

McCauley, D. E. 1991. Genetic consequences of local

population extinction and recolonization. Trends in Ecology and Evolution 6:5–8.

McClenaghan, L. R., M. H. Smith, and M. W. Smith. 1985. Biochemical genetics of mosquitofish. IV. Changes of allele frequencies through time and space. Evolution 39:451–460.

Mickevich, M. F., and M. S. Johnson. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Systematic Zoology 25:260–270.

Mihok, S., W. A. Fuller, R. P. Canham, and E. C. McPhee. 1983. Genetic changes at the transferrin locus in the red-backed vole (*Clethrionomys gapperi*). Evolution 37:332–340.

Mueller, L. D., L. G. Barr, and F. J. Ayala. 1985a. Natural selection vs. random drift: evidence from temporal variation in allele frequencies in nature. Genetics 111:517–554.

Mueller, L. D., B. A. Wilcox, P. R. Ehrlich, D. Heckel, and D. D. Murphy. 1985b. A direct assessment of the role of genetic drift in determining allele frequency variation in populations of *Euphydryas editha*. Genetics 110:495–511.

Nei, M., and F. Tajima. 1981. Genetic drift and estimation of effective population size. Genetics 98: 625–640.

Nygren, J. 1980. Allozyme variation in natural populations of field vole (*Microtus agrestis* L). III. Survey of a cyclically density-varying population. Hereditas 93:125–136.

Olivieri, I., D. Couvet, and P.-H. Gouyon. 1990. The genetics of transient populations: research at the metapopulation level. Trends in Ecology and Evolution 5:207–210.

Pamilo, P., and S. Varvio-aho. 1980. On the estimation of population size from allele frequency changes. Genetics 95:1055–1057.

Patton, J. L., and J. H. Feder. 1981. Microspatial genetic heterogeneity in pocket gophers: non-random breeding and drift. Evolution 35:912–920.

Pollak, E. 1983. A new method of estimating the effective population size from allele frequency changes. Genetics 104:531–548.

Powers, D. A., and A. R. Place. 1978. Biochemical genetics of *Fundulus heteroclitus* (L.). I.Temporal and spatial variation in gene frequencies of *Ldh-B, Mdh-A, Gpi-B,* and *Pgm-A*. Biochemical Genetics 16:539–607.

Redfield, J. A. 1973. Demography and genetics in colonizing populations of blue grouse (*Dendragapus obscurus*). Evolution 27:576–592.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rohlf, F. J., and F. L. Bookstein. 1987. A comment on shearing as a method for "size correction." Systematic Zoology 36:356–367.

Schaffer, H. E., D. Yardley, and W. W. Anderson. 1977. Drift or selection: a statistical test of gene frequency variation over generations. Genetics 87: 371–379.

Selander, R. K. 1975. Stochastic factors in the genetic structure of populations. Pp. 284–331 *in* G. F. Estabrook, ed. Proceedings of the Eighth International Conference on Numerical Taxonomy. W. H. Freeman, San Francisco.

Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinction. Theoretical Population Biology 12:253–262.

———. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16:393–430.

Smith, M. F., and J. L. Patton. 1984. Dynamics of morphological differentiation: temporal impact of gene flow in pocket gopher populations. Evolution 38:1079–1087.

Steiner, W. W. 1979. Genetic variation in Hawaiian *Drosophila*. VI. Seasonally-dependent gene changes in *Drosophila mimica*. Evolution 33:543–562.

Tamarin, R. H., and C. J. Krebs. 1969. *Microtus* population biology. II. Genetic changes at the transferrin locus in fluctuating populations of two vole species. Evolution 23:183–211.

Templeton, A. R. 1974. Analysis of selection in populations observed over a sequence of consecutive generations. Theoretical and Applied Genetics 45: 179–191.

Tomiuk, J., and K. Wohrmann. 1981. Changes of the genotype frequencies at the *MDH*-locus in populations of *Macrosiphum rosae* (L.) (Hemiptera: Aphididae). Biologisches Zentralblatt 100:631–640.

Wade, M. J., and D. E. McCauley. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution 42:995–1005.

Waples, R. S. 1989a. Temporal variation in allele frequencies: testing the right hypothesis. Evolution 43:1236–1251.

———. 1989b. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379–391.

———. 1990a. Temporal changes of allele frequency in Pacific salmon: implications for mixed-stock fishery analysis. Canadian Journal of Fisheries and Aquatic Sciences 47:968–976.

———. 1990b. Conservation genetics of Pacific salmon. II. Effective population size and the rate of loss of genetic variability. Journal of Heredity 81:267–276.

———. 1990c. Conservation genetics of Pacific salmon. III. Estimating effective population size. Journal of Heredity 81:277–289.

Waples, R. S., and D. J. Teel. 1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conservation Biology 4:144–156.

Watterson, G. A. 1982. Testing selection at a single locus. Biometrics 38:323–331.

Weinberg, J. R., and V. R. Starczak. 1988. Morphological differences and low dispersal between local populations of the tropical beach isopod, *Excirolana braziliensis*. Bulletin of Marine Science 42: 296–309.

———. 1989. Morphological divergence of eastern Pacific and Caribbean isopods: effects of a land barrier and the Panama Canal. Marine Biology 103: 143–152.

White, M., and G. E. Svendsen. 1990. Genic homogeneity among temporal populations of the eastern chipmunk (*Tamias striatus*). Canadian Journal of Zoology 68:2169–2173.

Whitlock, M. C., and D. E. McCauley. 1990. Some population genetic consequences of colony forma-

tion and extinction: genetic correlations within founding groups. Evolution 44:1717–1724.
Wilson, S. 1980. Analyzing gene-frequency data when the effective population size is finite. Genetics 95: 480–502.
Wright, S. 1940. Breeding structure of populations in relation to speciation. American Naturalist 74: 232–248.
Zuniga, R., R. Pena, and M. Clarke. 1985. Historia de vida y produccion de *Excirolana braziliensis* Richardson, 1912 (Isopoda: Cirolanidae). Estudios Oceanologicos 4:9–19.

Corresponding Editor: G. Vermeij