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Biogeographic Analysis of the Herpetofauna Separated by the Formation of the Strait of Gibraltar

Between 5.5 and 7.0 m.y. B.P. the formation of the Strait of Gibraltar allowed water from the Atlantic Ocean to fill the basin now known as the Mediterranean Sea. This event divided the amphibian and reptile fauna then inhabiting southern Spain and northern Morocco into allopatric and, presumably, conspecific faunas. Because the strait has continued from its formation to the present as a geographic barrier to the exchange of terrestrial vertebrates between Africa and Europe, this region and its native herpetofauna provide a natural laboratory in which various tenets of current biogeographic theory may be evaluated.

Electrophoretically obtained allozymic data allowed an empirical biogeographic analysis of the trans-Gibraltar herpetofauna. Estimates of accumulated genetic distance (Nei's D̂) varied from 0.0 to 0.6 between Spanish and Moroccan populations of 17 conspecific taxa. Further examination of these genetic data demonstrated that the rate of migration between these two continental populations was greater than or less than the estimated rate of mutation. European and African populations of several taxa traditionally presumed to be homogeneous were shown to be genically heterogeneous. Analysis of the distribution of alleles within and between continental populations suggests that the ability to maintain genetic continuity across the marine barrier varies from species to species.

No demonstrable correlation was found between degree of habitat specificity and frequency of occurrence of individuals, and the genic differentiation demonstrated by Spanish populations of each taxon.

Differential dispersal of species across a geographic barrier may lead to the formation of a novel terrestrial biota (Mayr 1963, McDowell 1978); or a vicariant event that fragments formerly contiguous populations may lead to species formation with the identical eventual result (Nelson & Platnick 1981). Savage (1982) compared and contrasted the tenets of dispersal and vicariance hypotheses of biogeographic analysis. Under the dispersal hypothesis, biotas are shaped by differential dispersal across barriers, whereas the vicariance hypothesis advocates the evolution of biotas as a result of the formation of geographic barriers to gene exchange. Data supporting both hypotheses are available; Savage (1982) suggested that both dispersal and vicariance played major roles in the evolution of the Central American herpetofauna.

Once separated by geographic barriers, how rapidly do populations

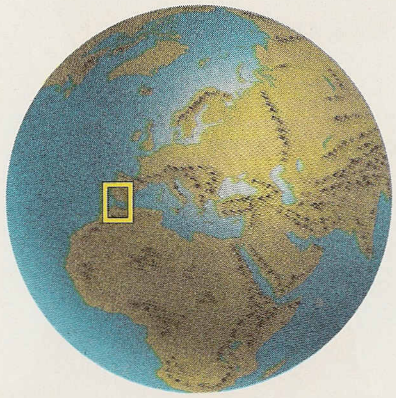

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Figure **1.** *Localities in Spain and Morocco from which specimens were collected. The single Moroccan specimen of* Coronella girondica *was collected from Oukaïmedene, some 540 km southwest of Tanger. Arrows show surface currents through the strait (after U. S. Naval Oceanographic Office 1965:Figure I-23).*

become genically differentiated? Few data bear on this point for at least two reasons: The barrier must have become absolutely effective recently enough to allow the study of population-level changes; and from the time of its formation the barrier must have continued to be effective and to prohibit genetic exchange between disjunct populations.

Between 2 and 5 million years ago, closure of the oceanic channel separating nuclear Central America from Panama partitioned marine fishes into Pacific and Atlantic components (Lloyd 1963, Woodring 1966). Vawter et al. (1980) examined interocean genetic divergence among five species of fishes believed to be conspecific and 10 species currently divided into five congeneric, morphologically very similar species pairs having one member in each ocean. Four conspecific pairs and two congeneric pairs were also examined for intraoceanic genic differentiation. Estimates of interoceanic genetic divergence (expressed as Nei's *D* units [Nei 1971]) between conspecific (0.13 to 0.36) and congeneric (0.14 to 0.32) taxa demonstrated equivalent ranges. In contrast, estimates of intraoceanic genetic divergence between two conspecifics (0.01 and 0.03) and four congenerics (between 0.01 and 0.03) were both equivalent and an order of magnitude lower than interoceanic divergence (Vawter et al. 1980:Tables 1 and 2). While these data have been challenged (Lessios 1981), they remain the most comprehensively reported zoogeographic analyses using genetic information.

Another recently formed, extant geographic barrier is the Strait of Gibraltar. This narrow (14 to 20 km) and deep (935 m) body of saline water was formed between 5.5 and 7.0 million years ago when rising ocean levels allowed water from the Atlantic Ocean to fill the Mediterranean Basin (for a review of events see Ager 1980, Busack & Hedges 1984, Fernix et al. 1967, Hsü 1983). Estimates of the age of the strait are drawn from the study of gravitational anomalies and paleomagnetism (Gaibar-Puertas 1973), Mediterranean evaporites (Hsü et al. 1977), sedimentation rates (Stanley et al. 1975), Foraminifera (Tjalsma 1971), and Ostracodes (Benson 1976). These estimates are concordant, and this precise dating of the separation of Europe from Africa provides an opportunity to study evolution in the resulting independent populations of terrestrial organisms.

Six species of amphibians and 18 species of reptiles are usually interpreted as having conspecific populations on either side of the Strait of Gibraltar (Bons 1973). These species are presumed to have inhabited northern Morocco and southern Spain before the formation of the Strait of Gibraltar. With the possible exception of the aquatic and saline-tolerant Spanish terrapin *Mauremys leprosa,* each would theoretically have been divided into two allopatric populations when the strait formed.

The results of an electrophoretic examination of the species included within this trans-Gibraltar assemblage of reptiles and amphibians are reported here. Divergence in taxa affected by the strait would be expected to be related to the degree of effectiveness of the strait as a barrier. Taxa that had differentiated geographically before the formation of the Strait of Gibraltar would be expected to exhibit more genetic change than taxa that had not yet differentiated. Electrophoretic analysis of genic differentiation in this amphibian and reptile assemblage provides a means by which such taxa may be classified. Electromorphs present in one population but lacking in another are an additional means of estimating gene exchange. The barrier's effect can be assessed by analyzing the frequency of occurrence of such electromorphs and the geographic distributions of the populations in which they occur.

Microevolutionary events subsequent to the vicariant event, especially those influencing the number, extent, and periodicity of bottlenecks, as well as the long-term effective sizes of the populations under consideration, must also be accounted for in an analysis of this fauna. Data relevant to the population histories of these taxa must be inferred from current phenomena. A species living among a broad range of habitats today may have been more broadly distributed and less affected by environmental perturbation in the past than a specialized one. (In fact, only broad-niched amphibians and reptiles may have survived historic events in this region [Busack & Jaksić 1982a].) Likewise, a widely distributed, populous species is more likely to be available to repopulate an area than a narrowly distributed species represented by few individuals. Knowing the current abundance and the degree of habitat specificity of a species permits speculation concerning the level of genic differentiation that existed between local populations in the past (Endler 1977 and references cited therein).

In the present analysis, ecological data were combined with information on actual genetic differentiation within and between amphibian and reptile populations from northern Morocco and southern Spain. This study was initiated to clarify the contribution molecular data can be expected to make in studies of biogeography.

Conspecific Spanish and Moroccan Taxa Considered in this Analysis

AMPHIBIA
Caudata
  Salamandridae
    Pleurodeles waltl
Anura
  Discoglossidae
    Discoglossus pictus
  Bufonidae
    Bufo bufo
  Hylidae
    Hyla meridionalis

REPTILIA
Testudines
  Emydidae
    Mauremys leprosa
Squamata
  Amphisbaenia
    Amphisbaenidae
      Blanus cinereus
  Lacertilia
    Gekkonidae
      Tarentola mauritanica
    Lacertidae
      Acanthodactylus erythrurus
      Lacerta lepida
      Podarcis hispanica
      Psammodromus algirus
    Scincidae
      Chalcides chalcides
  Serpentes
    Colubridae
      Coluber hippocrepis
      Coronella girondica
      Macroprotodon cucullatus
      Malpolon monspessulanus
      Natrix maura

Materials and Methods

Genic Differentiation

Between March and July 1982 and March and April 1983, 17 sets of presumably conspecific (Bons 1973) amphibians and reptiles (listed to the right) were collected from the areas of Spain and Morocco identified in Figure 1. In addition to these taxa, *Rana perezi* (Anura: Ranidae) and *Pelobates cultripes* (Anura: Pelobatidae) of Spain, and their apparent sister taxa *R. "saharica"* and *P. varaldii* of Morocco were also collected for analysis. (The systematic status of Moroccan populations of *Rana* is uncertain [Hemmer et al. 1980, Uzzell 1982].) All taxa affected by the formation of the Strait of Gibraltar should have been separated at least 5.5 to 7.0 million years, whether or not they can be regarded currently as conspecifics or as sister species.

Specimens were killed in the field and samples of heart and liver removed, frozen, and stored in liquid nitrogen (−196°C). In the laboratory, tissues were stored frozen (−76°C) until they were analyzed electrophoretically (between two and 12 months). For each animal, tissue samples were pooled and 35 proteins assayed using standard horizontal starch-gel electrophoresis (11.5% hydrolyzed starch, Sigma Chemical Co.) and histochemical staining (Ayala et al. 1972, Harris & Hopkinson 1976, Selander et al. 1971). Genetic interpretations of allozymic data are based on criteria developed by Selander et al. (1971); the electrophoretic protocol and allozymic data are presented in detail elsewhere (Busack 1985:Tables 1 and 2, Appendix I).

Various proteins — such as nonspecific esterases, some enzymes not involved in complex metabolic pathways, and plasma proteins — are known to accumulate electrophoretically detectable substitutions more rapidly than other proteins. Rapidly evolving proteins theoretically have different electrophoretic mobilities after 5 to 6 million years of separation; further accumulation of genetic distance reflects changes in more slowly evolving proteins (Sarich 1977). The effect of differential rates of evolution in various proteins is neutralized in this analysis by critically examining variation within and among only those 25 proteins common to all species sampled (26 presumed genetic loci that could be reliably scored [Busack 1985:Appendix I]).

Nei (1973) has shown that two genetically identical populations may demonstrate genetic distances larger than zero when the sample sizes are small. Because many of the sample sizes used in the analysis reported here are smaller than desirable (fewer than five individuals per locality), estimates of heterozygosity and genetic distance were computed using Nei's unbiased method (1978). These formulae theoretically can be applied to any sample size, and produce unbiased estimates of heterozygosity and genetic distance.

Samples analyzed first as separate geographic populations within Spain and Morocco were later combined to exemplify populations representative of either Spain or Morocco. Allele distribution and frequencies (Busack 1985:Appendix II) were used to compute values (Nei 1978) and standard errors (Nei 1971) for estimates of Nei's $\hat{D}$. Genetic distances between Spanish or Moroccan populations — geographically separated by 14 to 20 km — are available for many of the species (Busack 1985:Appendix III); these are the "controls" that enable comparisons of terrestrial versus overwater effects.

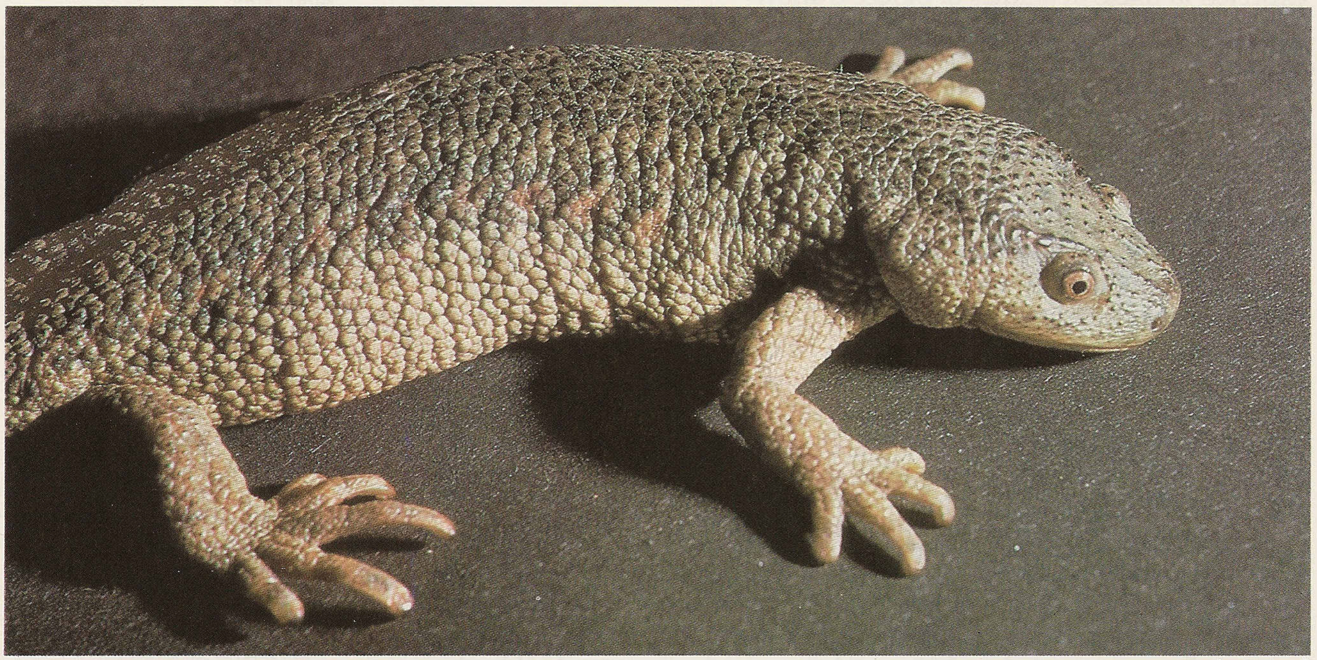

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Figure **2.** Pleurodeles waltl, *Puerto Real, Spain.*

Habitat Diversity

Apparent differences in species' use of habitat may indicate which factors precipitated genic differentiation within and between continental populations. If a species that is currently distributed throughout widely differing habitat types were similarly distributed in the past, it probably would have been less subject to environmental perturbations than a species whose range of habitat use was narrowly restricted. If such ecological differences affect levels of genic differentiation among conspecific populations, an analysis that correlates ecology with genic differentiation may be useful.

Collections made within Cádiz province, Spain, from 1969 to 1972 and 1982 to 1983 provide locality data for the taxa found in southern Spain (Busack 1977). Because these data contain several duplications of a single locality for a single taxon, and because it was only necessary to determine the habitat generality or specificity of each taxon, only the first appearance of a taxon at a specific locality was included in the calculations. A single occurrence of a taxon at any site was sufficient to document its presence in this habitat type; *Mauremys leprosa,* because of its exclusively aquatic habit, is not included in these computations. Table 1 shows the number of localities contributing to the determination of habitat diversity and evenness of representation in the remaining 16 species.

The Instituto Nacional de Investigaciones Agronómicas (1971) provided a 1:200 000-scale map in which 14 categories of land use within Cádiz province were identified. In addition to areas of specific agricultural pursuit (rice, cotton, vineyards, etc.), this map included unimproved areas in categories (agriculturally unproductive [talus, salt flats], pastoral areas of low elevation, chapparal, etc.). By locating the 1790 available collecting sites on this map, it was possible to assign each collecting locality to a specific land-use (i.e., habitat) category.

The habitat category of each locality was recorded, the number of times each category appeared was tabulated, and the numerical representation of each of the 14 habitat categories was computed. An index based on these data enabled computation of the habitat diversity (Brillouin's H′) and evenness (J) for each species (Table 1) (Busack 1980, Poole 1974). Species with high diversity values were considered habitat generalists, while those with low values were considered specialists; an evenness value of 1.0 (perfectly balanced) indicated a habitat generalist that used all 14 habitat categories with equal frequency.

Table *1*. Spearman's Coefficient of Rank Correlation Between Ecological Factors and the Number of Alleles Differentiating the Two Geographically Closest Populations Within Southern Spain

Taxon	Alleles no. (rank)	Habitat Diversity H′ (rank)	Habitat Diversity J (rank)	Number of Localities (rank)	Abundance in Collection (rank)
Discoglossus pictus	12 (3.5)	1.7 (13.5)	0.7 (12.5)	127 (10.5)	178 (12.0)
Rana perezi	15 (1.5)	1.5 (10.0)	0.6 (8.0)	140 (12.0)	201 (14.0)
Bufo bufo	12 (3.5)	1.3 (6.5)	0.5 (4.0)	127 (10.5)	95 (8.0)
Hyla meridionalis	11 (5.5)	1.7 (13.5)	0.7 (12.5)	301 (15.0)	179 (13.0)
Blanus cinereus	11 (5.5)	1.0 (2.5)	0.5 (4.0)	24 (3.0)	13 (2.0)
Tarentola mauritanica	7 (10.0)	1.0 (2.5)	0.4 (1.5)	116 (8.0)	171 (11.0)
Acanthodactylus erythrurus	7 (10.0)	0.7 (1.0)	0.4 (1.5)	14 (1.0)	146 (9.0)
Lacerta lepida	3 (15.0)	1.5 (10.0)	0.6 (8.0)	184 (14.0)	73 (7.0)
Podarcis hispanica	15 (1.5)	1.2 (4.5)	0.5 (4.0)	149 (13.0)	154 (10.0)
Psammodromus algirus	4 (13.5)	1.4 (8.0)	0.6 (8.0)	88 (7.0)	207 (15.0)
Chalcides chalcides	10 (7.0)	1.2 (4.5)	0.6 (8.0)	15 (2.0)	14 (3.0)
Coluber hippocrepis	8 (8.0)	1.6 (12.0)	0.7 (12.5)	48 (5.0)	19 (4.0)
Macroprotodon cucullatus	5 (12.0)	1.3 (6.5)	0.6 (8.0)	27 (4.0)	12 (1.0)
Malpolon monspessulanus	4 (13.5)	1.8 (15.0)	0.8 (15.0)	119 (9.0)	32 (5.0)
Natrix maura	7 (10.0)	1.5 (10.0)	0.7 (12.5)	65 (6.0)	72 (6.0)
Spearman's coefficient (r_s):		0.09	0.20	– 0.25	– 0.25
Probability:		$\geqslant 0.50$	0.50	>0.20	>0.20

H′ = Brillouin's measure for habitat diversity, J = evenness of habitat use

Abundance Estimates

Apparent differences in abundance in this herpetofaunal assemblage may also indicate which factors precipitated genic differentiation within and between continental populations. If the numerical representation of a species affects the level of genic differentiation between conspecific populations, an analysis that correlates abundance with genic differentiation should demonstrate this relationship.

The systematic sampling routine by which collections were made within Cádiz province from 1969 to 1972 provided one estimate of species abundance. The total number of specimens representing each species collected during this period indicates the relative abundance of each taxon in southern Spain (Busack 1977). With this method, museum specimens were directly counted but specimens that were recorded but not collected were ignored. Because *Acanthodactylus erythrurus* was intensively studied at one site (Busack 1976, Busack & Jaksić 1982b, Pough & Busack 1978), only the number collected during the first visit to that site is included. A second index, provided by summing the number of sites (localities) at which each taxon was observed, involves assigning a value of one to every locality at which a species was observed. This index provided a control for sampling bias: Many road-killed snakes were not salvaged as museum specimens; frogs were identified when they called from inaccessible areas but were not collected; and many lizards were observed but escaped capture.

Migration Across the Strait of Gibraltar

If the Strait of Gibraltar is an effective barrier to gene exchange among all trans-Gibraltar amphibians and reptiles, the rate of mutation should be greater than the rate of migration in all taxa. Maximum possible migration rates between Spanish and Moroccan populations, consistent with electrophoretically determined polymorphisms, can be calculated with the formula:

$$I = m/(m + \nu)$$

where I is the genetic identity between populations, ν the mutation rate, and m the level of migration (Nei 1975:194). This formula includes the assumption that migration rate greatly exceeds mutation rate, but it provides an estimate for maximum possible migration rate when ν and m are approximately equal.

The 95% confidence intervals for the rate of mutations per locus per generation in *Drosophila* have been calculated as 3.7×10^{-7} to 5.3×10^{-6} (Mukai & Cockerham 1977). Because mutation rates have not been calculated for any of the taxa examined in this study, these confidence intervals were used to approximate the value of ν in estimates for m in the Mediterranean herpetofauna. *Drosophila* and humans have approximately the same calculated rate of mutation (1.8×10^{-6} vs. 2×10^{-6} [Mukai & Cockerham 1977, Nei 1975, respectively]); thus, the similar rate despite organismal differences makes the use of these data for estimating amphibian and reptile mutation rates seem reasonable.

Statistical Analysis

If the calculated genetic distances are normally distributed, this would support the null hypothesis of no relationship between the timing of the formation of the Strait of Gibraltar and the genetic distances of the herpetofauna. If the genetic distances are not normally distributed — if in fact they are clumped — there may be several reasons. Clumping around zero, for instance, might indicate either that the strait is not an effective barrier (i.e., that migration is common) or that there has been no genic differentiation between populations since their formation. D'Agostino's test was used to determine how far intercontinental genetic distances departed from normality (Zar 1974).

Spearman's coefficient of rank correlation was used to evaluate the relationship of the variety of habitats a species occupies (habitat diversity), the evenness with which it occupies those habitats, and the relative abundance of a species to the allelic differentiation between populations within southern Spain (Table 1) (Zar 1974). Significance levels for all statistical tests were set at $p < 0.05$ (two-tailed test).

Results

Gorman & Renzi (1979) empirically assessed the effect of sample size on estimates of heterozygosity and genetic distance. They found that genetic distance estimates based on 22 loci from one individual deviated from estimates derived from 22 loci and 25 individuals by a Nei distance ≤ 0.1; and that the *D* calculated from data provided by only one individual usually overestimated the *D* calculated from data provided by the full population. This analysis further suggested that heterozygosity estimates based on two individuals were generally within 2.5% of values estimated for the full population.

When the computation of Nei's *D* was based on only 15 of the 22 loci examined among samples of 25 individuals per population, values of *D* deviated further from those derived from 22 loci than did values of *D* based on 22 loci for only one individual per population. Further investigation, which also involved manipulating the number of considered loci, led to the conclusion that both heterozygosity and genetic distance estimates were more severely affected by the number of loci sampled than by the number of individuals sampled. Within the trans-Gibraltar herpetofauna, the difference between estimates of heterozygosity obtained by examining 26 loci and those obtained by examining between 31 and 41 loci was, on average, 2%. Estimates of polymorphism varied about 10%, with 18 of 38 estimates being reduced, 17 being increased, and three not changing at all (Table 2).

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Figure **3.** Bufo bufo, *Facinas, Spain.*
4. Hyla meridionalis, *Chechaouene, Morocco.*

In a study such as this one, where technical and financial constraints limit the number of loci that can be evaluated over the entire faunal comparison, caution must be exercised in the interpretation of genetic distances. For example, when the full complement (between 31 and 41) of reliably scored loci was compared with 26 common loci, genetic distance estimates increased in five, decreased in five, and remained unchanged in nine (Table 2, Figure 5). Among those taxa demonstrating reduced genetic distance with increased loci were the highly differentiated species pairs *Pelobates cultripes–P. varaldii* (Nei's $\hat{D}$ decreased from 0.86 to 0.79) and *Rana perezi–R. "saharica"* (from 0.59 to 0.55), and the intercontinentally well-differentiated *Chalcides chalcides* (from 0.60 to 0.45). Two other species, *Bufo bufo* and *Coronella girondica*, exhibited about a 0.04 genetic distance unit reduction (from 0.16 and 0.17 to 0.11 and 0.14, respectively) when additional loci were considered.

The genetic distances separating Spanish and Moroccan populations of *Tarentola mauritanica, Psammodromus algirus,* and *Malpolon monspessulanus* increased from about 0.0 to about 0.1 as a result of the addition of loci. African and European *Lacerta lepida* demonstrated a similar increase, from about 0.1 to about 0.2, and the estimated distance separating continental populations of *Blanus cinereus* increased from about 0.5 to about 0.6 when more than 26 loci were considered. The standard error of a computed genetic distance is also directly related to the number of loci surveyed; therefore increasing the number of loci surveyed does increase confidence in any given genetic distance. However, for purposes of the analysis at hand, the effect of differential rates of evolution in various proteins may only be controlled by using data contributed by the 26 common loci. While such a restriction does not significantly alter the overall result (Figure 5), it does affect comprehension of the genetic structure of some of the taxa.

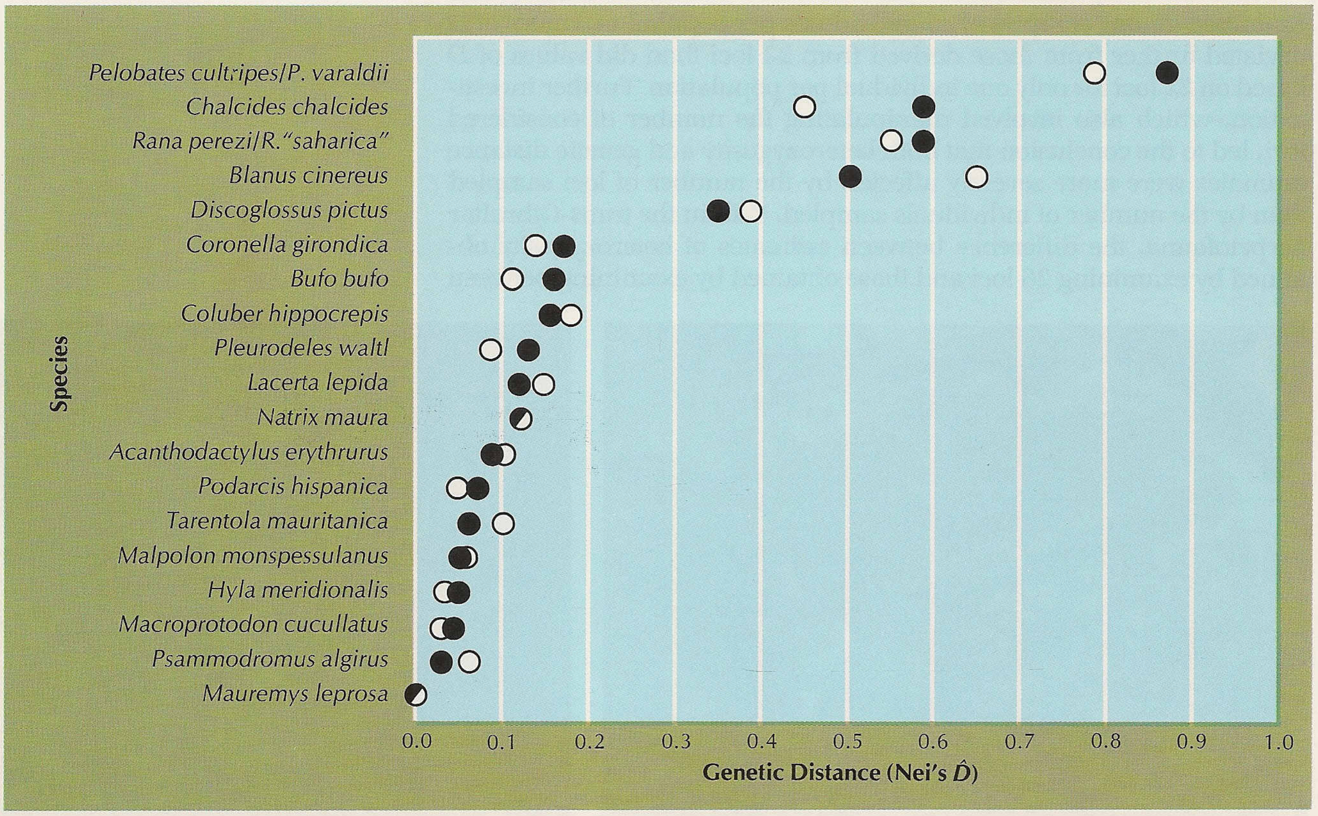

***Figure 5.** Nei's (1978) $\hat{D}$ values based on 26 (closed circles) and between 31 and 41 (open circles) loci for Spain–Morocco comparisons. See Table 2 for the actual values.*

Some populations were represented by fewer than five individuals (Table 2) and limited sample sizes have been shown to overestimate genetic distances (Gorman & Renzi 1979). If this tendency applies to the taxa examined here, the estimated differentiation between Spanish and Moroccan *Bufo, Lacerta, Coluber, Coronella,* and *Malpolon* essentially disappears. Moroccan *Pelobates* and *Chalcides* are each represented by two specimens that differ greatly from Spanish individuals (Busack 1985:Appendix II). But even if genetic distances were overestimated by 0.1 unit, the general conclusions would not be significantly affected.

The genetic distances and associated 95% confidence limits separating Spanish from Moroccan populations of 21 amphibian and reptile species are presented in Table 2.

Table 2. **Intercontinental Genetic Distance ($\hat{D}$) with Associated Confidence Limits and Estimated Mean Heterozygosity per Locus with Percentage of Loci Polymorphic**

Taxon	$\hat{D}$	Loci Fixed Differences	Specimens		Loci Examined	95% Confidence Limits	Mean Heterozygosity		Loci Polymorphic (%)	
			Spain	Morocco			Spain	Morocco	Spain	Morocco
Pelobates cultripes & P. varaldii	0.86	13	5	2	26	0.41 to 1.32	0.16	0.12	38.5	19.2
	0.79	**15**	**5**	**2**	**32**	**0.41 to 1.17**	**0.17**	**0.02**	**40.6**	**15.6**
Chalcides chalcides	0.60	8	8	2	26	0.25 to 0.94	0.09	0.10	30.8	15.4
	0.45	**10**	**8**	**2**	**35**	**0.20 to 0.70**	**0.09**	**0.08**	**31.4**	**11.4**
Rana perezi & R. "saharica"	0.59	5	10	13	26	0.24 to 0.93	0.23	0.20	57.7	73.1
	0.55	**6**	**10**	**13**	**31**	**0.25 to 0.85**	**0.21**	**0.20**	**58.1**	**71.0**
Blanus cinereus	0.51	9	10	9	26	0.19 to 0.82	0.10	0.14	26.9	30.8
	0.65	**13**	**10**	**9**	**33**	**0.32 to 0.98**	**0.14**	**0.15**	**33.3**	**30.3**
Discoglossus pictus	0.35	6	7	10	26	0.10 to 0.60	0.19	0.20	42.3	53.9
	0.39	**9**	**7**	**10**	**34**	**0.16 to 0.62**	**0.16**	**0.16**	**38.2**	**44.1**
Coronella girondica	0.17	2	4	1	26	0.01 to 0.34	0.12	0.04	30.8	3.8
	0.14	**3**	**4**	**1**	**37**	**0.02 to 0.27**	**0.10**	**0.03**	**24.3**	**2.7**
Coluber hippocrepis	0.16	3	5	1	26	0.00 to 0.32	0.12	0.00	26.9	0.0
	0.18	**5**	**5**	**1**	**39**	**0.04 to 0.31**	**0.12**	**0.00**	**28.2**	**0.0**
Bufo bufo	0.16	3	10	3	26	0.00 to 0.32	0.13	0.13	38.5	23.1
	0.11	**3**	**10**	**3**	**37**	**0.00 to 0.22**	**0.10**	**0.13**	**30.0**	**24.3**
Lacerta lepida	0.13	2	11	4	26	– 0.01 to 0.27	0.08	0.12	30.8	23.1
	0.15	**3**	**11**	**4**	**39**	**0.02 to 0.28**	**0.12**	**0.11**	**38.5**	**23.1**
Pleurodeles waltl	0.13	2	5	10	26	– 0.01 to 0.28	0.05	0.05	11.5	11.5
	0.09	**2**	**5**	**10**	**40**	**– 0.01 to 0.18**	**0.04**	**0.04**	**10.0**	**10.0**
Natrix maura	0.12	2	9	12	26	– 0.02 to 0.25	0.11	0.09	34.6	19.2
	0.12	**2**	**9**	**12**	**36**	**0.00 to 0.23**	**0.12**	**0.11**	**36.1**	**27.8**
Acanthodactylus erythrurus	0.09	2	10	9	26	– 0.03 to 0.21	0.05	0.15	26.9	38.5
	0.11	**3**	**10**	**9**	**37**	**0.00 to 0.22**	**0.07**	**0.19**	**27.0**	**46.0**
Podarcis hispanica	0.07	0	8	14	26	– 0.03 to 0.18	0.21	0.26	50.0	65.4
	0.05	**0**	**8**	**14**	**33**	**– 0.03 to 0.13**	**0.18**	**0.22**	**42.4**	**57.6**
Tarentola mauritanica	0.05	0	7	10	26	– 0.04 to 0.14	0.13	0.09	30.8	23.1
	0.10	**1**	**7**	**10**	**37**	**0.00 to 0.21**	**0.15**	**0.10**	**32.4**	**27.0**
Malpolon monspessulanus	0.05	1	5	1	26	– 0.04 to 0.14	0.09	0.00	23.1	0.0
	0.06	**2**	**5**	**1**	**36**	**– 0.02 to 0.13**	**0.09**	**0.00**	**19.4**	**0.0**
Hyla meridionalis	0.05	0	15	10	26	– 0.04 to 0.13	0.13	0.11	38.5	38.5
	0.04	**0**	**15**	**10**	**34**	**– 0.03 to 0.10**	**0.10**	**0.10**	**32.4**	**32.4**
Macroprotodon cucullatus	0.04	0	10	5	26	– 0.04 to 0.12	0.07	0.12	15.4	26.9
	0.03	**0**	**10**	**5**	**41**	**– 0.02 to 0.09**	**0.06**	**0.13**	**14.6**	**26.8**
Psammodromus algirus	0.03	0	9	13	26	– 0.04 to 0.09	0.06	0.06	19.2	23.1
	0.06	**1**	**9**	**13**	**35**	**– 0.02 to 0.14**	**0.07**	**0.08**	**22.9**	**25.7**
Mauremys leprosa	0.00	0	6	13	26	0.00	0.02	0.01	7.7	3.8
	0.00	**0**	**6**	**13**	**37**	**0.00**	**0.04**	**0.14**	**10.8**	**10.8**

Estimates based on 26 (regular type) *and between 31 and 41* (**bold-faced type**) *loci.*

Distribution of Alleles

While differences in the distribution of genetic loci that are fixed between populations indicate reduced reproductive contact in the past, differences in allelic frequency may also indicate restricted genetic exchange. Distribution of alleles at each locus varies both between continental populations and between populations residing on the same continent. The appearance or disappearance of individual alleles in this trans-Gibraltar fauna may be regarded as independent events. Each allele therefore becomes a separate character whose presence in or absence from a population can be scored (Buth 1984). Alleles apparently present only in Spanish populations are designated "European" alleles; alleles apparently present only in Moroccan populations are designated "African"; and alleles present in only one of two geographically close local populations on each continent are designated "local" alleles.

While no "fixed" differences were identified among comparisons of either Spanish or Moroccan populations, several were identified when Spanish populations were compared with Moroccan populations. A fixed difference between continental populations must, by definition, contain at least one African and one European allele. Not all European and African alleles, however, represent loci at which fixed differences were identified. European and African alleles not represented in loci demonstrating fixed differences are enumerated in Table 3 in an effort to further differentiate between the populations.

Genetic distances between continents vary from being about equal to being six times as large as genetic distances within continents in those taxa for which Spanish or Moroccan local population comparisons are available (i.e., all taxa except *Pelobates* and *Coronella*, Table 4). For the 10 taxa listed in the upper third of Table 4, the level of genic differentiation (Nei's $\hat{D}$) between Spanish and Moroccan populations is no greater than the distance between local Spanish or Moroccan populations. The number of local alleles in Spain ranges from three in *M. leprosa* and *L. lepida* to 15 in *P. hispanica*. In Morocco this number is similar and ranges from one in *M. leprosa* to 15 in *P. hispanica*. When European and African alleles identified in these taxa are combined, the sums range from one in *M. leprosa* to 19 in *P. hispanica* (Table 3).

Table 3. Distribution of African, European, and Local Alleles

Taxon	Alleles (Specimens)		No. Local Alleles Differentiating Two Closest Local Populations	
	Europe	Africa	Spain (Specimens)	Morocco (Specimens)
Discoglossus pictus	17 (7)	23 (10)	12 (5–2)	20 (5–5)
Pelobates cultripes & P. varaldii	22 (5)	17 (2)	—	—
Rana perezi & R. "saharica"	16 (10)	20 (13)	15 (5–5)	15 (5–5)
Chalcides chalcides	18 (8)	13 (2)	10 (5–3)	—
Blanus cinereus	14 (10)	16 (9)	11 (5–5)	13 (5–3)
Podarcis hispanica	5 (8)	14 (14)	15 (5–3)	15 (5–5)
Acanthodactylus erythrurus	6 (10)	12 (9)	7 (5–5)	12 (5–4)
Bufo bufo	11 (10)	7 (3)	12 (5–5)	6 (2–1)
Coluber hippocrepis	11 (5)	3 (1)	0 (3–2)	—
Natrix maura	8 (9)	5 (12)	7 (5–3)	6 (3–8)
Coronella girondica	10 (4)	3 (1)	—	—
Hyla meridionalis	7 (15)	5 (10)	11 (5–5)	5 (5–5)
Lacerta lepida	6 (11)	6 (4)	3 (5–5)	8 (2–1)
Tarentola mauritanica	6 (7)	5 (10)	7 (5–2)	5 (5–5)
Macroprotodon cucullatus	4 (10)	6 (5)	5 (5–5)	6 (2–2)
Pleurodeles waltl	4 (5)	6 (10)	—	5 (5–5)
Malpolon monspessulanus	6 (5)	1 (1)	4 (3–1)	—
Psammodromus algirus	2 (9)	4 (13)	2 (5–4)	5 (5–3)
Mauremys leprosa	1 (6)	0 (13)	3 (4–1)	1 (3–7)

Among those taxa with higher intercontinental than intracontinental genetic distances (middle third of Table 4), populations found within Spain exhibit between zero (in *C. hippocrepis*) and 15 (in *R. perezi*) local alleles. The number of local alleles found in Moroccan species varies between five in *P. waltl* and 20 in *D. pictus*. Summed European and African alleles in these taxa range from 10 characteristic of *P. waltl* to 41 characteristic of *D. pictus* (Table 3).

Migration Rates

Estimates of the maximum possible rates of migration between populations of these trans-Gibraltar species also suggest a heterogeneous pattern within this herpetofauna (Table 5). Four species (top section of Table 5) demonstrate rates of migration that are approximately equivalent to estimated rates of mutation in each case. The calculated maximum rate of migration between *P. cultripes* and *P. varaldii* broadly overlaps the estimated mutation rate, and lower limit estimates of migration rate encompass only slightly the upper limits of mutation rate in eight species (middle section of Table 5). The estimated rate of migration of the remaining six species (bottom section of Table 5) is higher than the estimated rate of mutation.

Ecological Variables

Rank correlation coefficients (r_s) between 0.09 and 0.25 ($p > 0.2$ to $p \geqslant 0.5$) were derived from comparing ecological variables with the number of local alleles identified in Spanish populations. No simple relationship exists between the abundance of a species, or between the species' demonstrated diversity of habitat-type usage, and the number of local alleles identified between populations in southern Spain (Table 2).

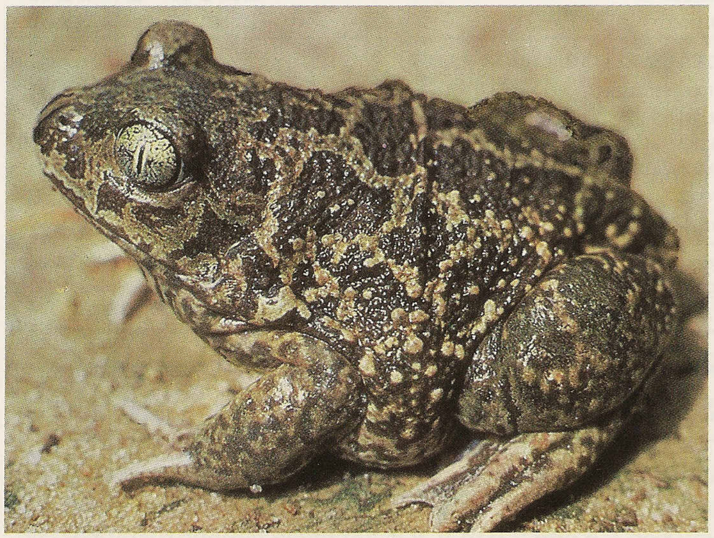

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Figure **6.** Pelobates cultripes, *Benalup de Sidonia, Spain.* **7.** Coluber hippocrepis, *Tanger, Morocco.*

Table 4. **Intercontinental Versus Intracontinental Genetic Distances**

Taxon	Within Spain	Within Morocco	Between Spain and Morocco
EQUIVALENT VALUES FOR INTERCONTINENTAL AND INTRACONTINENTAL $\hat{D}$ VALUES			
Lacerta lepida	0.05	0.09	0.13
Natrix maura	0.05	0.08	0.12
Acanthodactylus erythrurus	0.01	0.10	0.09
Podarcis hispanica	0.08	0.10	0.07
Tarentola mauritanica	0.06	0.02	0.05
Hyla meridionalis	0.05	0.04	0.05
Malpolon monspessulanus	0.02	—	0.05
Macroprotodon cucullatus	0.01	0.03	0.04
Psammodromus algirus	0.01	0.02	0.03
Mauremys leprosa	0.08	0.00	0.00
INTERCONTINENTAL GREATER THAN INTRACONTINENTAL $\hat{D}$ VALUES			
Chalcides chalcides	0.05	0.17	0.60
Rana perezi & R. "saharica"	0.13	0.12	0.59
Blanus cinereus	0.13	0.21	0.51
Discoglossus pictus	0.15	0.17	0.35
Coluber hippocrepis	0.11	—	0.16
Bufo bufo	0.08	0.06	0.16
Pleurodeles waltl	—	0.01	0.13
NO INTRACONTINENTAL VALUES AVAILABLE			
Pelobates cultripes & P. varaldii	—	—	0.86
Coronella girondica	—	—	0.17

Table 5. Maximum Possible Migration Rates

MIGRATION RATES ABOUT EQUAL MUTATION RATES	
Discoglossus pictus	8.83×10^{-7} to 1.26×10^{-5}
Blanus cinereus	5.62×10^{-7} to 8.05×10^{-6}
Chalcides chalcides	4.59×10^{-7} to 6.58×10^{-6}
Rana perezi & *R. "saharica"*	4.64×10^{-7} to 6.65×10^{-6}
MIGRATION RATES BROADLY OVERLAP MUTATION RATES	
Pelobates cultripes & *P. varaldii*	2.69×10^{-7} to 3.85×10^{-6}
Podarcis hispanica	5.03×10^{-6} to 1.00×10^{-4}
Acanthodactylus erythrurus	3.84×10^{-6} to 1.00×10^{-4}
Natrix maura	2.95×10^{-6} to 4.23×10^{-5}
Lacerta lepida	2.73×10^{-6} to 3.91×10^{-5}
Pleurodeles waltl	2.58×10^{-6} to 3.70×10^{-5}
Bufo bufo	2.18×10^{-6} to 3.12×10^{-5}
Coluber hippocrepis	2.18×10^{-6} to 3.12×10^{-5}
Coronella girondica	1.95×10^{-6} to 2.79×10^{-5}
MIGRATION RATES EXCEED MUTATION RATES	
Macroprotodon cucullatus	8.84×10^{-6} to 1.00×10^{-4}
Hyla meridionalis	7.69×10^{-6} to 1.00×10^{-4}
Malpolon monspessulanus	7.07×10^{-6} to 1.00×10^{-4}
Tarentola mauritanica	6.67×10^{-6} to 1.00×10^{-4}
*Mauremys leprosa**	3.70×10^{-3} to 5.30×10^{-2}
Psammodromus algirus	1.40×10^{-5} to 2.00×10^{-4}

These data were computed from the formula $I = m / (m + v)$ (Nei 1975:194) in which the 95% confidence intervals of v were assumed to be 3.7×10^{-7} and 5.3×10^{-6}, respectively.

**Genetic distance of 0.0001 was substituted for the value of 0.000 actually obtained.*

Discussion

A geographic barrier (the Strait of Gibraltar) separating the amphibian and reptile taxa of Africa from those of Europe has presumably been in place since between 5.5 and 7.0 m.y. B.P. Nineteen genetic distances estimating the amount of divergence that has accumulated in 21 members of this trans-Gibraltar herpetofauna range from a Nei's $\hat{D}$ of about 0.0 to a Nei's $\hat{D}$ of about 0.9. When presented graphically, the 19 genetic distances describe a curvilinear relationship not significantly different from a normal distribution (Figure 5; D'Agostino's $D = 0.25$, $p < 0.01$).

Stochastically varying change in particular protein molecules averages out to a constant rate of change over a number of different molecules through time (Carlson et al. 1978, Sarich 1977). In the absence of data to the contrary, the conservative assumption is made here that the rate of accumulation of genetic change within both Spanish and Moroccan populations of all taxa has remained relatively constant since the formation of the Strait of Gibraltar. Under this assumption, intercontinental genetic distances greater than intracontinental genetic distances reflect the genetic change that has accumulated between European and African populations as a result of the formation of the Strait of Gibraltar. Intercontinental $\hat{D}$ estimates were compared with intracontinental $\hat{D}$ estimates for each taxon: Equivalent values were demonstrated for 10 taxa (topmost section of Table 4); seven taxa exhibited intercontinental $\hat{D}$ estimates greater than intracontinental $\hat{D}$ values; and two had to be disregarded because no intracontinental values were available.

Current distributions of European and African alleles indicate that rates of change within Spanish and Moroccan populations of taxa comprising this trans-Gibraltar herpetofauna may not have been, or may not now be, equivalent on each continent (Table 3). Among those species demonstrating equivalent intercontinental and intracontinental genetic distances, the number of alleles that have the potential of differentiating between Spanish and Moroccan populations ranges from only one in *M. leprosa* to 19 in *P. hispanica* (Tables 3 & 4).

The distribution of alleles in taxa that have higher intercontinental than intracontinental Nei's $\hat{D}$ values demonstrates that between 10 and 39 alleles are found only in Spanish or in Moroccan populations of these taxa. While the genera *Discoglossus* (seven European, 23 African, and about 16 local alleles) and *Rana* (16 European, 20 African, and 15 local alleles) appear to be highly variable both within and between continents, *Pleurodeles* is substantially less fragmented (four European, six African, and five local alleles) (Table 3). Substantially more European and African than local alleles are identified in these taxa. This latter difference alone indicates that the Strait of Gibraltar is a stronger barrier to gene exchange than any intracontinental terrestrial barrier presented to *P. waltl, D. pictus, R. perezi–R. "saharica," B. cinereus, C. chalcides,* and *C. hippocrepis* (Table 4, middle section). If the accumulation of African or European alleles is simply a function of the amount of time that has elapsed since the populations were last in reproductive contact, then the range in the number of African and European alleles may reflect the differential effect of the barrier on each taxon, the degree of genic fragmentation within each taxon prior to the formation of the strait, the differing rates of change among taxa, or a combination of all these factors.


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Figure 8. Macroprotodon cucullatus, *Kenitra prefecture, Morocco.*

Among the Gibraltar herpetofauna, those taxa demonstrating similar genetic distances within and between continents do not necessarily demonstrate a uniform distribution of African or European alleles. Moroccan populations of *A. erythrurus,* for instance, have 12 of the 18 alleles found to differentiate between Spanish and Moroccan populations, while Spanish populations have only six. If, before the formation of the strait, Moroccan and Spanish *Acanthodactylus* were one genically uniform population, these data suggest that Moroccan populations have accumulated change at about twice the rate of Spanish conspecifics. Spanish populations of *H. meridionalis* exhibit 11 local alleles, while

Moroccan populations exhibit only five (Table 3). Spanish *H. meridionalis* therefore appear less homogeneous than Moroccan *H. meridionalis*. If the taxa in this faunal assemblage were less than genically uniform at the time of the formation of the strait, if they evolved at different rates, or if they were subject to different rates of extinction (all possibilities which these data may support), then a single correlation between genetic distance and the time of this geological event is not appropriate.

Another source of variation within these data involves the determination of genetic distances and associated standard errors. Most of the taxa examined here are represented by fewer than 20 specimens; the "accuracy" of a genetic distance depends upon sample size, level of heterozygosity, and number of loci examined (Nei 1978). While Nei's formula for obtaining an unbiased estimate of heterozygosity is mathematically applicable to any sample size (Nei 1978), its biological appropriateness remains untested. Nevertheless, heterozygosity estimates derived by applying this formula to the full set of loci (between 31 and 41 loci) for each taxon varied by only about 2% from estimates derived from a 26-loci comparison (Table 2).

Nei (1978) suggested that a relatively large number of individuals be examined in those organisms in which average heterozygosity was > 0.1. Heterozygosity in 14 of the taxa examined was ≤ 0.1 (Table 2). Of these taxa, the Nei's $\hat{D}$ differentiating continental populations of the species represented by the largest number of individuals (*H. meridionalis* — 25 specimens, 15 from Spain and 10 from Morocco) exhibited 95% confidence limits between −0.04 and 0.13. The confidence limits for Nei's $\hat{D}$ differentiating continental populations of the species represented by the fewest individuals (*C. girondica* — five specimens, four from Spain and one from Morocco) were 0.01 to 0.34.

The 95% confidence limits surrounding the genetic distance estimates for the largest sample with high heterozygosity (*Rana*, 23% heterozygosity — 23 individuals, 10 from Spain and 13 from Morocco) were 0.24 to 0.93. The smallest sample exhibiting high heterozygosity (16%) was *Pelobates* (seven specimens, five from Spain and two from Morocco) and the 95% confidence limits for this two-species comparison were 0.41 to 1.32. The nature of the algorithm is such that the greater the average heterozygosity per locus, the more difficult it becomes to determine a narrowly bounded estimate of genetic distance. Levels of heterozygosity varied greatly among taxa examined in this project (Table 2) and those examined in others (Nevo 1978). For this reason alone, using Nei's genetic distances as indicators of elapsed time is inadvisable.

Had data on single reptile and amphibian species been used to estimate the elapsed time since the formation of the strait, estimates would have varied greatly depending upon the species selected and upon the calibration (Avise & Aquadro 1982). For example, use of the common reptile *P. hispanica* ($\hat{D} = 0.07$) and the common amphibian *D. pictus* ($\hat{D} = 0.35$) would have yielded respective estimates of 0 to 3.4 m.y. B.P. and 1.9 to 11.3 m.y. B.P. for the formation of the strait (calibration according to Carlson et al. 1978). The most extreme cases, of course, are those made by six taxa in which the computed Nei's $\hat{D}$ between Spanish and Moroccan populations was 0.0 (Table 2).

The degree to which one could accurately preselect those species most likely to be genically differentiated is limited. For example, in an earlier zoogeographical analysis of the fossil evidence and current distributions of these species, it was suggested that occasional rafting could be maintaining gene flow in what were considered to be undifferentiated populations (Busack 1977:294). This suggestion was based in part on extensive experience and knowledge of the life histories and ecology of this fauna. The aquatic turtle *Mauremys leprosa* is known to tolerate salinity well (Schoffeniels & Tercafs 1966) and is the most likely candidate for occasional transport. The colubrid snake *Natrix maura* inhabits the margins of creeks that are often flooded by heavy rains, and adult *Pleurodeles waltl* tolerate salinity and desiccation (Knoepffler 1962). *Discoglossus pictus* larvae transform normally in water with an elevated salt content (6 g/L, Knoepffler 1962:42) and *Bufo bufo* tadpoles have been recorded from brackish water (Hagström 1981). The ubiquitous tree frog (*Hyla meridionalis*) is an explosive breeder whose wet season habitats include virtually any flooded area, including coastal sand dunes. The current range of the gecko *Tarentola mauritanica* is known to reflect human-assisted transport (Martínez Rica 1974), and the habitat generality and abundance of *Psammodromus algirus* suggest that this lacertid lizard may also be a candidate for accidental transport. Based on their ecology and behavior, these eight species are least likely to be isolated completely from conspecifics by the strait.

The barrier would appear to be most effective against the frogs *Rana perezi* and *R. "saharica," Pelobates cultripes* and *P. varaldii;* the amphisbaenian *Blanus cinereus;* the skink *Chalcides chalcides;* and the lacertid lizard *Acanthodactylus erythrurus.* The fossorial nature of the amphisbaenian and the skink, and the habitat specificity of the lacertid lizard and the pelobatid frogs (sandy areas) render them unlikely prospects for rafting. The distribution and availability of fresh water is known to have influenced the distributions of several species of *Rana* (Pace 1974). Geographic isolation may be easily imposed on these aquatic frogs and the formation of the Strait of Gibraltar may have been an important factor in precipitating the derivation of *R. perezi* and *R. "saharica"* from a common ancestor.

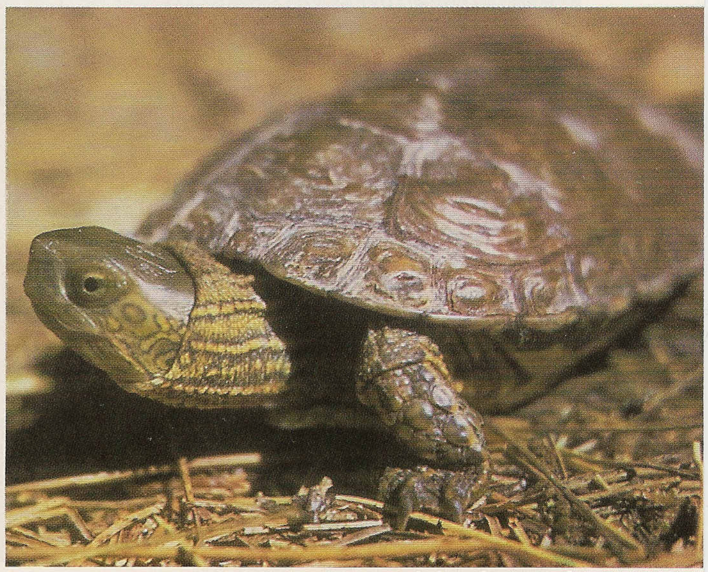

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Figure **9.** Blanus cinereus, *Kenitra prefecture, Morocco.* **10.** Mauremys leprosa, *Tétouan prefecture, Morocco.*

The probability of transport for the remaining taxa is difficult to assess. Juveniles of the lacertid lizard *Lacerta lepida* inhabit the bark of standing and fallen trees, while adults live under large boulders, in stone walls, and in the foundations of old buildings. The Spanish wall-lizard *Podarcis hispanica* appears equally at home in natural and manmade habitats. Snakes of the genera *Coluber, Coronella, Macroprotodon,* and *Malpolon* are also often encountered beneath the loose bark of fallen trees. Occasional transport as the result of flooding due to heavy seasonal rains is not difficult to envision for any of these taxa.

Precipitation in Quaternary Iberia and northern Africa fluctuated widely during the Pleistocene; although not as exaggerated, today's pattern of seasonal rainfall is probably reminiscent of the semiarid periods interspersed with periods of high precipitation characteristic of the past (Biberson 1970). Figure 1 illustrates today's normal direction and intensity of surface currents through the Strait of Gibraltar. During heavy rainfall, which causes severe flooding in northern Morocco, these currents increase and salinity might be temporarily reduced as heavy rains lie on the surface. Once upon the waters of the strait, occasional waifs could be washed by the currents into Spain's Bay of Algeciras (Figure 1). The possibility of migration (in the case of *Mauremys leprosa*) or inadvertent transport for these undifferentiated taxa appears real.

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Figure **11.** Malpolon monspessulanus, *Benalup de Sidonia, Spain.*

The genetic data derived from the present study greatly improve the possibility of assessing the effect of the Strait of Gibraltar on individual taxa. For example, species that could be considered good candidates for continued reproductive continuity—such as *P. waltl, D. pictus, B. bufo,* and *L. lepida*—are genically more differentiated, but *A. erythrurus* is not as differentiated as expected. Electrophoretically determined genetic identities allow an empirical assessment of the maximum possible rate of migration between Spanish and Moroccan populations. If taxa in which the computed maximum possible rate of migration exceeds the rate of mutation are not substantially affected by the barrier, then representatives of these taxa (Table 5, bottom section) may occasionally cross the strait. In the four taxa in which maximum migration rates are equivalent to mutation rates (Table 5, top section), it is difficult to assess the role the Strait of Gibraltar plays in the genetic structure. Migration rates broadly overlap mutation rates in nine taxa (Table 5, middle). Migration rate data based on genetic identities allow a more empirical means of assessing the role of the barrier, but not the question of which species, if any, are able to cross the strait.

A more direct and defensible estimate of the effect of the Strait of Gibraltar on this herpetofauna may be derived from an analysis of unshared alleles. One allele that is present in all sampled individuals within Spain, yet is absent from all sampled individuals within Morocco (or vice versa), indicates a cessation of gene flow. However, differing sample sizes seldom allow conclusive identification of such alleles. In this study, only those alleles present in the smaller sample (one or two specimens) and absent from the larger sample (several specimens) were considered to represent such alleles. From the data listed to the right, it is clear that reproductive continuity has been interrupted among certain representatives of the trans-Gibraltar fauna.

Prior to the formation of the Strait of Gibraltar the local distribution of each species was probably similar to its distribution today, and the range of environments each species encountered was more or less the same as that existing at present. If any local population is most likely to differentiate from the least accessible of neighboring populations, then the amount of genic differentiation between two populations should be proportional to the absolute amount of habitat change that has occurred over the distance between them. Genetic distances between local populations of no species on either continent are greater than those found between populations inhabiting different continents. However, genetic distances between continental populations of eight taxa are greater than those between populations inhabiting the same continent (Table 4). In addition, 13 taxa were found to exhibit alleles in all individuals of one continental population that were totally absent from the sample from the opposing continent. Sampling error may have misrepresented the actual distribution of some alleles, but in general these results suggest that the formation of the Strait of Gibraltar was the major physical barrier that precipitated differentiation.

If all taxa had begun genic differentation at the time the Strait of Gibraltar was formed, the genetic distances derived from intercontinental comparisons of all taxa would serve as independent calibrations of a taxon-specific molecular "clock." Genetic distances within the anurans range between 0.05 and 0.86; within the lizards, 0.03 and 0.59; and within the snakes, 0.04 and 0.17. These data suggest that, to be meaningful, any taxon-specific calibration of a molecular clock must be made at the generic or specific level. However, the meaningfulness of any clock concept rapidly diminishes with the increase in necessary calibrations.

Because of apparent differential, species-specific abilities to cross and maintain genetic continuity across a marine barrier, it is concluded here that the formation of the Strait of Gibraltar has provided a geographic barrier sufficient to precipitate (or perhaps maintain) differentiation in several amphibian and reptile taxa, while other taxa resident in the region are apparently able to cross the marine barrier. The variable degree of genetic differentiation among these taxa suggests that the population structure of each was affected differently by the environmental changes the whole region must have undergone (Tables 2 & 3).

In the only directly comparable study in which vertebrates were examined, the genetic distances (Nei's *D*) between Atlantic and Pacific populations of 15 fish taxa in Panama ranged from 0.1 to 0.4 (Nei 1971) and the investigators considered that averaging these distances produced an acceptable estimate of "community" genetic distance. This average distance was later accepted as confirmation of the applicability of the molecular clock hypothesis (Vawter et al. 1980). Reexamination of the data presented in Table 1 of Vawter et al. indicates that their use of differing numbers of loci and specimens, and lack of control over localities from which fish samples were collected may have confounded the

No. of Alleles Found Only in Spanish or Moroccan Populations

0 ALLELES
Hyla meridionalis
Mauremys leprosa
Podarcis hispanica
Psammodromus algirus
Macroprotodon cucullatus

1 ALLELE
Tarentola mauritanica
Acanthodactylus erythrurus
Malpolon monspessulanus

2 ALLELES
Coronella girondica

3 ALLELES
Bufo bufo
Coluber hippocrepis
Natrix maura

4 ALLELES
Pleurodeles waltl
Lacerta lepida

5 or more ALLELES
Discoglossus pictus
Blanus cinereus
Chalcides chalcides
Pelobates cultripes–P. varaldii
Rana perezi–R. "saharica"

results. The concordance of the averaged fish data with a clock-predicted genetic distance value may simply have been a coincidence.

As the genetic data obtained in this study clearly indicate, all taxa are not uniformly affected by the same geologic event. If all populations are conspecific yet not genically uniform before the event, if all do not accumulate change at the same rate, or if all are not similarly affected by the geographic barrier, the considered taxa will present differing levels of genic differentiation. Without reliable information regarding each of these factors, applying genetic distances to the timing of biogeographical events is at best speculative.

Geologic events that alter an area inhabited by populations of sexually reproducing organisms have a direct effect on these organisms. Without genetic information, any assessment of the effect this geologic event actually has is subjective. With genetic information, zoogeographic analyses become more empirical. Rather than presenting intelligently considered and constructed scenarios of what was and what is, biogeographers may now present conclusive evidence in support or refutation of a hypothesis. In the present study of the Gibraltar herpetofauna, several taxa previously considered to represent homogeneous entities in Spain and Morocco (Busack 1977) were found to be genetically heterogeneous. This finding suggests that a closer examination of the morphological similarities of each of these genically differentiated taxa is warranted and, in addition, sends a cautionary message to those whose mission it is to reconstruct evolutionary history.

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