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Historical biogeography, mitochondrial DNA, and allozymes of *Psammodromus algirus* (Lacertidae): a preliminary hypothesis

Stephen D. Busack[1], Robin Lawson[2]

Abstract. Pairwise sequence polymorphism in mitochondrial DNA and levels of differentiation among presumptive gene loci (expressed as Nei's $\hat{D}$) tend to be greater between populations separated by the Strait of Gibraltar than between populations inhabiting either Morocco or Spain. Ancestral *Psammodromus algirus* inhabiting Iberia and North Africa while the Strait of Gibraltar was being formed and stabilized (Miocene-Pliocene) evolved in association with physiogeographic change brought about by this barrier to gene exchange. Considered in units of genetic change per kilometer, mtDNA differentiation is greater in Morocco than in Spain, and allozyme differentiation is slightly greater than, or equal to, that in Spain, suggesting that *P. algirus* has a longer and more complex history in Morocco than in Spain.

Introduction

Found virtually throughout Iberia, along the Mediterranean coast of France, and across North Africa from southern Morocco to the northern half of Tunisia, *Psammodromus algirus* is often one of the most common reptiles encountered (Guillaume, 1997; Schleich et al., 1996:444-447). Geographic change involving the formation of the Strait of Gibraltar is fairly well understood (see Busack et al., 2005: fig. 5), but we are just beginning to understand diverse effects this cataclysmic series of events had on amphibian and reptile populations inhabiting the region. While no genetic change is apparent in populations of the stripe-necked turtle, *Mauremys leprosa*, on either side of the Strait of Gibraltar (Busack, 1986; Feldman and Parham, 2004: fig. 1), painted frogs in the genus *Discoglossus* (inferred from data in Martínez-Solano, 2004) and ribbed-newts in the genus *Pleurodeles* (Carranza and Arnold, 2004; Veith et al., 2004) likely differentiated as a result (either direct or indirect) of the formation of the Strait of Gibraltar. Harris et al. (2002) hypothesized that wall lizards in the genus *Podarcis* probably arrived in Iberia from North Africa through two transmarine dispersal events and Busack et al. (2005) further hypothesized that divergence between *Podarcis hispanica* and *P. vaucheri* was directly influenced by events associated with the formation of the Strait; Iberian populations of the fringe-toed lizard, *Acanthodactylus erythrurus*, were clearly separated from Moroccan populations by this event (Harris et al., 2004a). Chameleons, *Chamaeleo chamaeleon*, one gecko, *Tarentola mauritanica*, and the false smooth snake, *Macroprotodon brevis*, are considered recent human-driven introductions to Iberia from North Africa (Paulo et al., 2002; Harris et al., 2004b, c; Carranza et al., 2004, respectively).

In a previous investigation involving this event, Busack (1986: table 1) determined that among lizard taxa *Psammodromus algirus* and *Lacerta lepida* were least specialized with regard to habitat preference. Busack and Jaksić had earlier (1982:295 and table 2) found *P. algirus* inhabiting the Spanish province of Cádiz to be microclimate generalists that appeared to favor grassland or orchard-like (structurally similar to open, broad-leafed forest in unaltered situations) habitat; Guillaume (1997:303) noted that the species occurs only exceptionally on is-

1 - North Carolina State Museum of Natural Sciences, 11 West Jones Street, Raleigh, North Carolina 27601-1029, USA
e-mail address: steve.busack@ncmail.net
2 - Osher Foundation Laboratory for Molecular Systematics and Department of Herpetology, California Academy of Sciences, 875 Howard Street, San Francisco, California 94103-3009, USA
e-mail address: rlawson@calacademy.org

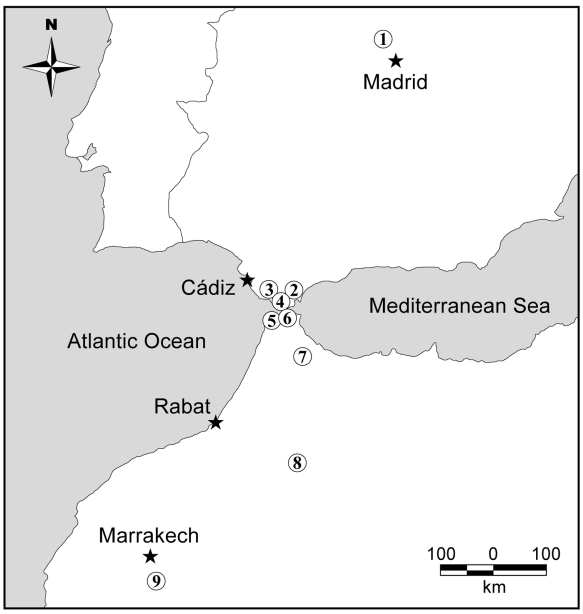

Figure 1. Study areas in Spain and Morocco. Specimens examined from the vicinity of Manzanares el Real (1), the Río Hozgarganta (2), Benalup de Sidonia (3), Facinas (4), Tanger and Cap Spartel (5), Ksar-es-Seghir (6), Chechaouèn (7), Âïn Leuh (8), and Oukaïmedèn (9).

Table 1. Primers used for *Psammodromus* DNA amplification and sequencing. Primers from the current study are named to indicate position of the 3′ nucleotide in the mitochondrial genome of *Eumeces egregious* (Kumazawa and Nishida, 1999).

Primer	Primer Sequence	Use	Location	Reference
L14910	5′-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3′	Amp.	tRNA-glu	de Queiroz et al., 2002
L14919	5′-AAC CAC CGT TGT TAT TCA ACT-3′	Amp./Seq.	tRNA-glu	Burbrink et al., 2000
L14761	5′-MTC HAA CAA CCC AAY MGG-3′	Seq.	Cyt *b*	This study
H14892	5′-TGC NGG KGT RAA KTT TTC-3′	Seq.	Cyt *b*	This study
H16064	5′-CTT TGG TTT ACA AGA ACA ATG CTT TA-3′	Amp./Seq.	tRNA-thr	Burbrink et al., 2000
H15149	5′-CCC TCA GAA TGA TAT TTG TCC TCA-3′	Seq.	Cyt *b*	Kocher et al., 1989
L4437b	5′-CAG CTA AAA AAG CTA TCG GGC CCA TAC C-3′	Amp./Seq.	tRNA-met	Kumazawa et al., 1996
L4411	5′-AAC CAA ACM CAM CTA CG-3′	Seq.	ND2	This study
H5877	5′-AAA CTA GKA GCC TTG AAA GCC-3′	Amp./Seq.	tRNA-trp	de Queiroz et al., 2002
PsamND4r	5′-ATG GTG GGT GTG GAT CAA TTG-3′	Amp./Seq.	ND4	This study
PsamND4f	5′-TAA CAA ACC TTA CTA ACA TAG-3′	Amp./Seq.	ND4	This study
Leu	5′-CAT TAC TTT TAC TTG GAT TTG CAC CA-3′	Amp./Seq.	tRNA-leu	Arévalo et al., 1994

lands. In a recent study, Diaz et al. (2000) found survival in *P. algirus* to be related to vegetation structure except in areas of forest fragmentation; forest fragments smaller than 90 ha led to extinction due to limited dispersal ability of the species. Civantos and Forsman (2000:68), in a principal component analysis, found low positive loadings on rock and negative loadings on bare soil for microhabitats selected by juveniles. These latter two analyses suggest *P. algirus* as an unlikely survivor of the type of habitat change likely precipitated by the formation of the Strait of Gibraltar.

Substantial biological information is available with regard to this relatively common lizard, but population structure remains poorly known (Pérez-Mellado, 1998; Schleich et al., 1996). While investigating genetic differentiation in reptile populations inhabiting both northern and southern shores of the Strait of Gibral-

Table 2. Protein systems examined by electrophoresis; enzymes arranged by Enzyme Commission number.

Protein (abbreviation)	Enzyme Commission Number	Electrophoretic Conditions
General Proteins (Gp)	–	B
Alcohol dehydrogenase (Adh)	1.1.1.1	A
Glycerol-3-phosphate dehydrogenase (G3pdh)	1.1.1.8	D
L-iditol dehydrogenase (Iddh)	1.1.1.14	D
L-Lactate dehydrogenase (Ldh)	1.1.1.27	F
Malate dehydrogenase (Mdh)	1.1.1.37	F
Malate dehydrogenase (Nadp+) (Mdhp)	1.1.1.40	F
Isocitrate dehydrogenase (Idh)	1.1.1.42	E
Phosphogluconate dehydrogenase (Pgdh)	1.1.1.44	E
Glucose-6-phosphate dehydrogenase (G6pdh)	1.1.1.49	D
Aldehyde dehydrogenase (Aldh)	1.2.1.3	F
Glutamate dehydrogenase (Gtdh)	1.4.1.2	H
Superoxide dismutase (Sod)	1.15.1.1	D
Aspartate aminotransferase (Aat)	2.6.1.1	D
Hexokinase (Hk)	2.7.1.1	G
Creatine kinase (Ck)	2.7.3.2	G
Adenylate kinase (Ak)	2.7.4.3	G
Esterase (non specific) (Est)	3.1.1.-	B
Esterase (non specific) (Est-D)	3.1.1.-	B
Acid phosphatase (Acp)	3.1.3.2	G
Fructose-bisphosphatase (Fbp)	3.1.3.11	D
N-Acetyl-β-glucosaminidase (βga)	3.2.1.30	G
L-Leucylglycylglycine (Pep-B)	3.4.-.-	C
Dipeptidase I, L-Leucyl-L-Alanine (La)	3.4.13.11	B
Proline dipeptidase (Pep-D)	3.4.13.9	B
Adenosine deaminase (Ada)	3.5.4.4	A
Aconitase hydratase (Acoh)	4.2.1.3	E
Mannose-6-phosphate isomerase (Mpi)	5.3.1.8	E
Glucose-6-phosphate isomerase (Gpi)	5.3.1.9	F
Phosphoglucomutase (Pgm)	5.4.2.2	E

A. Histidine, pH 7.8 gel & electrode buffer (Harris and Hopkinson, 1976), 150v/3h.
B. LiOH A + B, pH 8.2 gel & LiOH A, pH 8.1 electrode buffer (Selander et al., 1971), 300v/3h.
C. Poulik, pH 8.7 gel & borate, pH 8.2 electrode buffer (Selander et al., 1971), 250v/3h.
D. Tris citrate II, pH 8.0 gel & electrode buffer (Selander et al., 1971), 130v/4h.
E. Tris citrate II, pH 8.0 + NADP gel & tris citrate II, pH 8.0 electrode buffer (Selander et al., 1971), 130v/4h.
F. Tris citrate III, pH 7.0 gel & electrode buffer (Ayala et al., 1972), 180v/3h.
G. Tris citrate III, pH 7.0 + 15% glycerine gel & tris citrate III, pH 7.0 electrode buffer (Ayala et al., 1972), 180v/3h.
H. Tris EDTA Borate I, pH 8.0 gel & electrode buffer (Selander et al., 1971), 200v/4h.

tar, we undertook analyses of specimens previously collected in Spain and Morocco and supplemented these data with those from an unpublished allozyme study of *Psammodromus* from this region (fig. 1). In some cases, the same specimens provided data for each data set. We present this preliminary synthesis of mtDNA and allozyme data as a supplement to our understanding of population structure and biogeographic history in *P. algirus.*

Material and Methods

One *Podarcis muralis* (the outgroup specimen) and thirty-two *Psammodromus algirus* collected in Spain and Morocco (fig. 1; see Specimens Examined for precise locality data) were euthanized in the field, and samples of heart and liver were removed, frozen, and stored in liquid nitrogen (−196°C). Tissues were transferred to a freezer (−76°C) in the laboratory, and used in allozyme analysis (heart and liver) seven to 15 months later or in DNA analysis approximately 20 years later. Each series of specimens from a single locality was collected during the same time period; 14 *P. algirus* provided mtDNA data and 25 *P. algirus* provided allozyme data. Eight *P. algirus* (MVZ 178364

and 178390 from Morocco; MNCN 11940-11943, 11957 and 11959 from Spain) provided data for both mtDNA and allozyme analyses.

DNA Analysis

Tissue was digested for 3-4 h at 65°C with constant motion in 2 ml of lysis buffer (Tris HCl 100 mM at pH 8.0, EDTA 50 mM at pH 8.0, NaCl 10 mM, SDS 0.5%) containing 60 μg of proteinase K per ml. Extraction twice with phenol/$CHCl_3$ at pH 7.3, then once with $CHCl_3$, followed digestion. DNA was precipitated from the aqueous layer with 2.5 volumes of pure ethanol and the precipitated DNA was then washed in 80% ethanol, dried and redissolved in TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0).

Template DNA for the polymerase chain reaction (PCR) was prepared by diluting stock DNA with TE buffer to give spectrophotometric absorption readings between 0.2 A and 0.7 A at A260. Mitochondrial DNA was amplified from template DNA in 100 μl reactions using a hot start method in a thermal cycler with a 7-min denaturing step at 94°C followed by 40 cycles of denaturing for 40 sec at 94°C, primer annealing for 30 sec at 46°C and elongation for one min at 72°C with a final 7-min elongation step at 72°C. PCR products were purified using Promega Wizard® PCR Preps DNA Purification System (Promega, Madison, WI, USA) according to manufacturer's instructions.

Cycle sequencing was performed on PCR products using the Big Dye® (Perkin-Elmer, Norwalk, CT) reaction premix for 50 cycles of 96°C for 10 sec, 45°C for 5 sec, and 60°C for four min. The nucleotide sequence was determined by using an ABI model 3100 Genetic Analyzer (Applied Biosystems, Norwalk, CT, USA). Oligonucleotide primers for amplification and sequencing were taken from the literature or designed for this project and are listed in table 1.

The entire cytochrome *b* gene (1143 bps) was amplified using primers L14910 and H16064. The sequence of single stranded DNA was obtained by using primers L14761, H14892 and H15149 for cycle sequencing. If ambiguous sites were found both DNA strands were sequenced using primers L14919 and H16064 in addition to those mentioned above.

Genes for the two subunits of NADH dehydrogenase were amplified and sequenced. The complete subunit 2 (ND2; 1038 bps) was amplified using primers L4437b and H5877 and sequence was obtained by using primers L4411 and H5877. A portion of subunit 4 (ND4; 709 bps) was amplified using primers PsamND4r, PsamND4f and leu; sequences of both strands of DNA were obtained by cycle sequencing with primers PsamND4r, PsamND4f and leu.

Nucleotide sequences were aligned using the program Sequencher™ version 4.0 (Gene Codes Corp., Ann Arbor, MI, USA). All sequences are deposited in GenBank (AY234145, 234155, 234166; DQ 150362-150391).

Because sequence data were not available from all genes from all specimens, we used both maximum parsimony (MP) and maximum likelihood (ML), executed in PAUP* version 4.0b4a (Swofford, 2000), to recover phylogenetic information from each gene separately and in combination (see de Queiroz et al., 2002, regarding the efficacy of this procedure). MP and ML analyses were conducted using the heuristic search mode with 1000 random stepwise addition replicates, tree-bisection-reconnection (TBR) branch swapping, all characters treated as unordered, and all character state changes weighted equally. Support for clades in 1,000 pseudoreplicates in each analysis was evaluated by nonparametic bootstrapping (Felsenstein, 1985) using the heuristic search mode with simple stepwise addition and TBR branch swapping.

Allozyme analysis

Tissue samples were pooled for each animal. Proteins were separated electrophoretically in horizontal starch gels (11.5% hydrolyzed starch, Sigma Chemical Co.) and localized by standard histochemical staining procedures (Ayala et al., 1972; Harris and Hopkinson, 1976; Selander et al., 1971; table 2).

Allozymic data for each protein system were obtained through "side by side" comparisons of Spanish and Moroccan material, and genetic interpretations of these data were based on criteria developed by Selander et al. (1971). Multiple loci within a protein system were numbered with "1" designating the most anodally migrating set of allelic products. Alleles of a locus were lettered, with "a" representing the most anodally migrating product (table 3). The unbiased minimum genetic distance between populations ($\hat{D}$), recommended by Nei (1978) for comparisons utilizing small sample sizes was computed from allele frequencies using algorithms provided by Nei (1978).

Alleles at each variable locus were then re-coded (with "1" being substituted for "a" to represent the most anodally migrating product) for further examination by Classical Discriminant Function Analysis (DFA). Population of origin (see fig. 1) was considered the grouping variable, alleles were considered predictors, and all groups were considered equal.

Miscellaneous

Airline distances between localities from which samples were collected were computed from 1:1,000,000 scale road maps published by Michelin for Morocco (1977, Number 169) and by Firestone Hispania for Spain (1969, España y Portugal).

Data distributions were tested for normality with the Kolmogorov–Smirnov test; normally distributed data were assessed for correlation using Pearson's product-moment procedure. We used 10,000 replicates of the Mantel Test (Sokal and Rohlf, 1998:813-819) to assess correlation among airline distances, Nei genetic distances and *p*-distances. The computer-based package written by Bonnet and Van de Peer (2001) provided the Mantel Test, SYSTAT 11.00.01(Systat Software, Inc., 2004) provided other statistical procedures. Statistical significance was assumed when $\alpha = 0.05$; all reported probabilities are those of committing a Type I error in a two-tailed test.

Table 3. Allele frequencies in *Psammodromus algirus*. Population numbers (in parentheses) coincide with designations in Fig. 1.

	Manzanares el Real	Benalup de Sidonia	Facinas	Tanger	Ksar-es-Seghir	Chechaouèn
Population:	(1)	(3)	(4)	(5)	(6)	(7)
N*	3	4	5	5	3	5
H**	0.03	0.04	0.09	0.08	0.03	0.05
% Polymorphic	5.71	8.57	20.0	20.0	5.71	14.3
Loci:						
Acp1	a	b	a(0.20) b(0.80)	b	b	a(0.20) b(0.80)
Acp2	a	b	b	b	b	b
Aldh	a	a(0.50) b(0.50)	a(0.60) b(0.40)	a	a	a
Est	a(0.333) c(0.667)	c	c(0.60) d(0.40)	b(0.20) c(0.80)	c	c
G6pdh	c	c(0.875) a(0.125)	c	b(0.50) d(0.50)	b(0.667) d(0.333)	b(0.20) d(0.80)
Hk	b	b	a(0.60) b(0.40)	b	b	b
Idh1	a	b	b(0.80) c(0.20))	b(0.20) d(0.80)	b(0.333) c(0.667)	d
Idh2	b	b	a(0.20) b(0.80)	b(0.40) c(0.60)	b	c
Iddh	a	a	a	a(0.80) b(0.20)	a	a
La	a(0.333) c(0.667)	c	c	b(0.20) c(0.80)	c	b(0.20) c(0.80)
Ldh2	b	a(0.25) b(0.75)	a(0.20) b(0.80)	a(0.20) b(0.80)	b	a(0.20) b(0.80)
Mdhp	a	a	a	a	a	a(0.80) b(0.20)

* Number of animals sampled per protein. **Average heterozygosity for all proteins (Nei, 1978).

Results

mtDNA

Complete cytochrome *b* sequences from 6 specimens provided 1,143 total (855 constant, 288 variable, and 38 parsimony-informative) characters. The MP analysis identified 144 possible rearrangements, and the score of the best tree was 326; the ML analysis identified 60 distinct data patterns, the best tree having a − ln likelihood of 2623.99. Complete ND2 sequences from 11 specimens provided 1038 total (717 constant, 321 variable, and 59 parsimony-informative) characters. The MP analysis identified 1,742 possible rearrangements, and the score of the best tree was 362; the ML analysis identified 76 distinct data patterns, but the − ln likelihood was unavailable due to missing data and/or ambiguities. Partial ND4 sequences from 13 specimens provided 709 total (505 constant, 204 variable, and 53 parsimony-informative) characters. The MP analysis identified 6,881 rearrangements, and the score of the best tree was 240; the ML analysis identified 77 distinct data patterns, but the − ln likelihood was unavailable due to missing data and/or ambiguities.

Because nodal configurations of all MP and ML trees generated separately from cytochrome *b*, ND2, and ND4 were concordant (data not shown), we combined data from all genes, with gaps treated as missing data, for further interpretation and discussion (fig. 2). In total, 2,890 aligned nucleotide positions (2,074 constant, 816 variable, and 163 parsimony-informative) were considered. The heuristic search employed

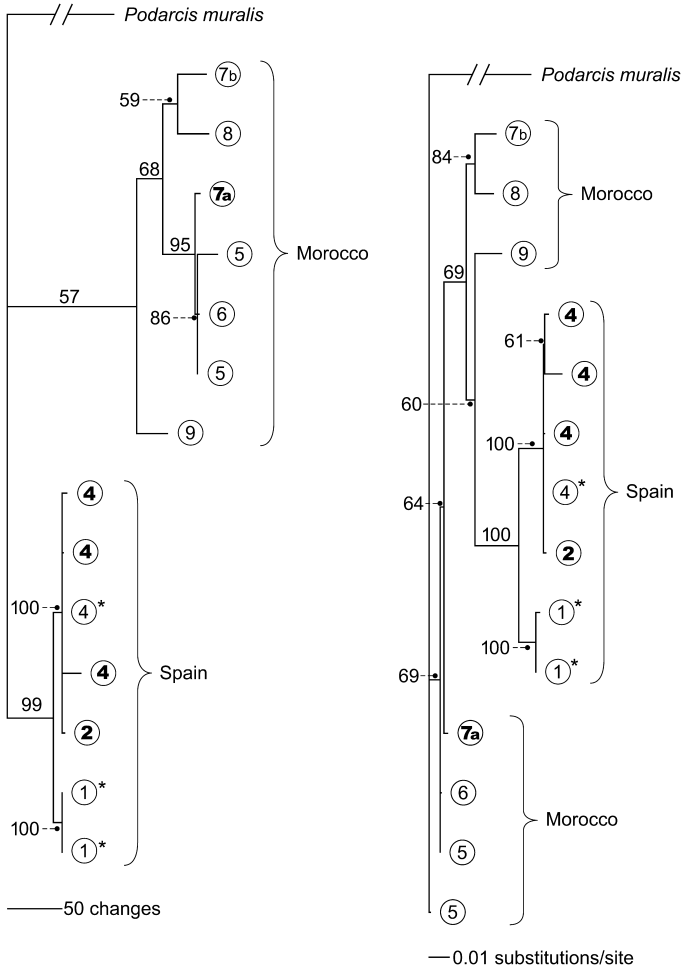

Figure 2. Maximum-parsimony (left) and maximum likelihood (right) trees derived from combined Cytochrome *b*, ND2, and ND4 gene sequences and rooted on *Podarcis muralis*. Numbers above nodes indicate bootstrap support (>50%, 1000 replicates). Numbers in circles correspond to locality designations in fig. 1; boldface indicates placement based on ND2 and ND4 only, * indicates ND4 only.

for MP analysis tried 59,720 rearrangements, with the resulting length of the best tree equal to 959 (consistency index [CI] = 0.90, homoplasy index [HI] = 0.10; excluding uninformative characters, CI = 0.68, HI = 0.32, retention index = 0.79, rescaled CI = 0.72). The ML analysis identified 220 distinct data patterns and 1901 rearrangements, and the − ln likelihood of the best tree was 7723.90.

Relationships between *p*-distance and geographic distance within Morocco are statistically significant for cytochrome *b* ($n = 10$, $r = 0.92$, $P \ll 0.05$), ND2 ($n = 16$, $r = 0.83$, $P \ll 0.05$) and ND4 ($n = 16$, $r = 0.87$, $P =\ll 0.05$); with all genes combined $n = 16$, $r = 0.87$, and $P =\ll 0.05$ (see tables 4 and 5). Because the Mantel test requires a minimum of five comparisons, we were not able to assess this relationship within Spain but we have no *a priori* reason to expect this relationship not to be significant within Spain as well.

When comparisons were made between *p*-distances and geographic distances involving only localities in which the formation of the Strait of Gibraltar was likely to have had a direct influence (between 2 + 4 and 5, 6, 7a, 7b, and

Table 4. Pairwise percent differences (mean ± SE, *n*) in Cytochrome *b* (above diagonal; **boldface** indicates values predicted from mean ND4 values [see text]) and pairwise percent differences calculated with Cytochrome *b*, ND2 and ND4 sequences combined (below diagonal; see also fig. 2). Where available, intrapopulational comparisons (*italics*) serve as diagonal place markers; numbers in parentheses correspond to locality designations in fig. 1.

	Manzanares el Real	Facinas & Río Hozgarganta	Tanger & Cap Spartel	Ksar-es-Seghir	Chechaouèn		Âïn Leuh	Oukaïmedèn
Locality	(1)	(2 + 4)	(5)	(6)	(7a)	(7b)	(8)	(9)
(1)	***0.21, 1*** 0.14, 1	**2.06**	**4.59**	**4.53**	**4.77**	**4.53**	**4.04**	**3.92**
(2 + 4)	2.24 ± .03, 10	– 0.43 ± 0.13, 10	**4.74**	**4.68**	**4.68**	**4.19**	**3.95**	**4.31**
(5)	5.14 ± .05, 4	5.26 ± 0.12, 10	*0.18, 1* 0.62, 1	0.18 ± .09, 2	**0.39**	2.10 ± 0.09, 2	2.71 ± 0.0, 2	3.15 ± 0.09, 2
(6)	5.07 ± .07, 2	5.03 ± 0.13, 5	0.35 ± 0.31, 2	–	**0.33**	2.10, 1	2.80, 1	3.15, 1
(7a)	5.30 ± .07, 2	4.90 ± 0.16, 5	0.80 ± 0.45, 2	0.35, 1	–	**2.89**	**2.64**	**3.25**
(7b)	5.09 ± .07, 2	4.88 ± 0.16, 5	2.70 ± 0.28, 2	2.46, 1	2.72, 1	–	2.10, 1	2.98, 1
(8)	4.52 ± .07, 2	4.25 ± 0.09, 5	2.74 ± 0.28, 2	2.49, 1	2.43, 1	1.87, 1	–	3.15, 1
(9)	4.36 ± .07, 2	4.72 ± 0.15, 5	3.19 ± 0.31, 2	2.91, 1	2.77, 1	2.94, 1	2.80, 1	–

Table 5. Pairwise percent differences (mean ± SE, *n*) in ND4 (above diagonal) and airline distances (km; below diagonal) between sampled populations. Where available, intrapopulational comparisons (**bold**) serve as diagonal place markers; numbers in parentheses correspond to locality designations in fig. 1.

	Manzanares el Real	Facinas & Río Hozgarganta	Tanger & Cap Spartel	Ksar-es-Seghir	Chechaouèn		Âïn Leuh	Oukaïmedèn
Locality	(1)	(2 + 4)	(5)	(6)	(7a)	(7b)	(8)	(9)
(1)	**0.14, 1**	2.28 ± 0.04, 10	5.22 ± 0.06, 4	5.15 ± 0.07, 2	5.43 ± 0.07, 2	5.15 ± 0.07, 2	4.58 ± 0.07, 2	4.44 ± 0.07, 2
(2 + 4)	525	**0.14 ± 0.03, 10**	5.40 ± 0.04, 10	5.33 ± 0.05, 5	5.33 ± 0.05, 5	4.76 ± 0.05, 5	4.48 ± 0.05, 5	4.90 ± 0.05, 5
(5)	575	50	**0.14, 1**	0.07 ± 0.07, 2	0.35 ± 0.07, 2	2.90 ± 0.07, 2	2.75 ± 0.07, 2	3.46 ± 0.07, 2
(6)	562	40	25	–	0.28, 1	2.96, 1	2.68, 1	3.39, 1
(7a)	641	117	90	79	–	3.24, 1	2.96, 1	3.67, 1
(7b)	641	117	90	79	0	–	2.40, 1	3.53, 1
(8)	878	317	310	282	206	206	–	3.10, 1
(9)	1122	595	546	563	508	508	308	–

8; table 4), there was no statistically significant association. For ND2 $r = 0.61$ ($n = 5$), for ND4 $r = 0.60$ ($n = 5$), and for all genes combined $r = 0.60$ ($n = 5$); all comparisons have P values greater than 0.05 but less than 0.10.

Figure 2 suggests that populations in Morocco may be partitioned geographically, with representation from northern (5 and 6, fig. 1) and southern (8 and 9, fig. 1) localities being present at Chechaouèn (fig. 1: 7). Museum of Vertebrate Zoology, University of California, Berkeley (MVZ) 178380 (fig. 2: 7a) appears closely aligned with localities 5 and 6 (fig. 2; tables 4 and 5), while MVZ 178381 (fig. 2: 7b) appears more closely aligned with locality 8 and, more distantly, with locality 9.

Within area samples from northern Morocco (5, 6, 7a; fig. 1), the 95% confidence limits for the mean number of transitions and transversions, respectively, for ND2 is −0.2 to 3.2 and 0.4-2.6 ($n = 6$, $\bar{x} = 1.5$ and 1.5); for ND4, 0.4-2.6 and 0.0 ($n = 6$, $\bar{x} = 1.5$ and 0.0), and for cytochrome *b*, −0.1 to 2.8 and 1.0-1.0 ($n = 3$,

$\bar{x} = 1.3$ and 1.0). Within samples from southern Morocco (7b, 8, 9; fig. 1), the 95% confidence limits for mean transitions and transversions, respectively, for ND2 is 11.3-26.7 and −0.4 to 6.4 ($n = 4$, $\bar{x} = 19.0$ and 3.0); for ND4, 7.0-24.96 and 1.5-9.1 ($n = 3$, $\bar{x} = 16.0$ and 5.3); and for cytochrome *b*, 14.3-43.0 and −0.2 to 5.5 ($n = 3$, $\bar{x} = 28.7$ and 2.7). The 95% confidence limits for mean transitions and transversions, respectively, for ND2 between clades in northern and southern Morocco is 19.5-21.8 and 2.2-3.8 ($n = 11$, $\bar{x} = 20.6$ and 3.0); for ND4, 14.9-17.6 and 4.8-6.5 ($n = 12$, $\bar{x} = 16.3$ and 5.7); and for cytochrome *b*, 23.4-30.2 and 2.8-4.5 ($n = 9$, $\bar{x} = 26.8$ and 3.7).

Within Spain, respective 95% confidence limits for mean transitions and transversions in southern area samples were 0.7-2.7 and 0.1-1.1 ($n = 7$, $\bar{x} = 1.7$ and 0.6) for ND2 and 0.6-1.4 and 0.0 ($n = 11$, $\bar{x} = 1.0$ and 0.0) for ND4. Between northern and southern area samples these values for ND4 were 14.6-15.6 and 1.0-1.0 ($n = 10$, $\bar{x} = 15.1$ and 1.0), respectively. We have neither ND2 comparisons involving the population in northern Spain, nor cytochrome *b* comparisons for any Spanish area samples.

The 95% confidence limits for mean number transitions and transversions, respectively, identified for ND2 between area samples in southern Spain (2, 4; fig. 1) and area samples in northern Morocco was 39.6-40.9 and 5.0-6.0 ($n = 16$, $\bar{x} = 40.3$ and 5.5) and for ND4 it was 29.5-30.2 and 8.0-8.0 ($n = 20$, $\bar{x} = 29.8$ and 8.0). Between area samples in southern Morocco and area samples in southern Spain these values, respectively, were 37.2-41.8 and 3.9-5.1 ($n = 12$, $\bar{x} = 39.5$ and 4.5) for ND2, and 24.7-26.5 and 7.0-8.3 ($n = 16$, $\bar{x} = 25.6$ and 7.6) for ND4.

When ND4 for one population in northern Spain (fig. 1: 1) is compared to that in area samples from northern Morocco, 95% confidence limits for mean transitions and transversions, respectively, were 29.4-31.1 and 7.0-7.0 ($n = 8$, $\bar{x} = 30.2$ and 7.0). When compared to area samples from southern Morocco, these values were 21.8-32.2 and 4.7-8.5 ($n = 5$, $\bar{x} = 27.0$ and 6.6), respectively.

Cytochrome *b* *p*-distances are strongly correlated with ND4 *p*-distances ($n = 10$, $r = 0.93$, $P < 0.001$) and with *p*-distances computed from all available sequences combined ($n = 11$, $r = 0.96$, $P < 0.001$); in *Psammodromus algirus*, either ND4 *p*-distances or *p*-distances computed from all available sequences combined could be used to calculate approximate cytochrome *b* *p*-distances. We used ND4 *p*-distances to approximate cytochrome *b* *p*-distances through application of the statistically significant ($r^2 = 0.92$; $F = 105.3$, df = 1,9, $P = 0.0$) regression formula "cytochrome *b* *p*-distance = 0.09 + 0.86 (ND4 *p*-distance)". While the constant in this formula (0.09) is not significantly different from zero ($t = 0.39$, $P = 0.7$), the slope is significant ($t = 10.26$, $P = 0.00$). When we used ND4 *p*-distances from eleven comparisons for which we also had cytochrome *b* *p*-distances, and compared formula-derived estimates to actual *p*-distances, the correlation was very strong ($r = 0.93$, $P \ll 0.001$); 95% confidence limits for the actual mean value for cytochrome *b* were 1.79% to 3.09% while those for the estimated mean value were 1.82% to 3.05%.

Allozymes

The products (55 alleles) of 35 presumptive gene loci were resolved; Aat1, Aat2, Acoh1, Acoh2, Ada, Adh, Ak, βga, Ck, Est-D, Fbp, Gp, Gpi, Gtdh, G3pdh, Ldh1, Mdh, Mpi, Pep-B, Pep-D, Pgdh, Pgm, and Sod were monomorphic. Allele frequency differences, coupled with the distribution of unique alleles, contribute to genetic distances (Nei's $\hat{D}$) from 0.013 to 0.105 between area samples within Spain, 0.007 to 0.061 within Morocco, and 0.038 to 0.128 between area samples inhabiting Spain and Morocco (table 6).

Ten alleles are unique to Spain and 8 are unique to Morocco, including the 2 alleles from each continent that comprise the one apparent fixed difference (G6pdh). Within Spain, the

Table 6. Nei (1978) unbiased genetic distance (above diagonal) and airline distance (km; below diagonal) between sampled populations. Numbers in parentheses correspond to locality designations in fig. 1.

	Manzanares el Real	Benalup de Sidonia	Facinas	Tanger	Ksar-es-Seghir	Chechaouèn
Locality	(1)	(3)	(4)	(5)	(6)	(7)
(1)	–	0.105	0.098	0.128	0.112	0.147
(3)	512	–	0.013	0.056	0.038	0.092
(4)	525	22	–	0.065	0.048	0.098
(5)	575	63	50	–	0.026	0.007
(6)	562	62	40	25	–	0.061
(7)	641	140	117	90	79	–

population at Manzanares el Real (fig. 1: 1) has at least 4 alleles not found at Benalup de Sidonia or Facinas (fig. 1: 3 and 4, respectively), and Benalup de Sidonia and Facinas have 5 alleles that may not be present at Manzanares el Real (table 3).

Discriminant function analysis of these data assigned 88% of individuals to the area samples from which they were derived. Individuals from area samples 1, 3, 6, and 7 (fig. 1) were correctly assigned with 100% accuracy. In Spain, 3 individuals from Facinas (fig. 1: 4) were correctly assigned but 2 individuals were incorrectly assigned to the population from Benalup de Sidonia (fig. 1: 3). In Morocco, 4 individuals from Tanger (fig. 1: 5) were correctly assigned but 1 individual was incorrectly assigned to the population from Chechaouèn (fig. 1: 7).

There is a statistically significant relationship between Nei's $\hat{D}$ and geographic distance ($n = 15$, $r = 0.90$, $P < 0.001$) overall, and between Nei's $\hat{D}$ and geographic distance when comparisons are intracontinental ($n = 7$, $r = 0.90$, $P < 0.005$). Comparisons between localities likely to have been affected by the formation of the Strait of Gibraltar (between 3 and 5, 6, and 7, and between 4 and 5, 6, and 7; table 6), however, demonstrate the highest degree of correlation ($n = 6$, $r = 0.96$, $P < 0.001$).

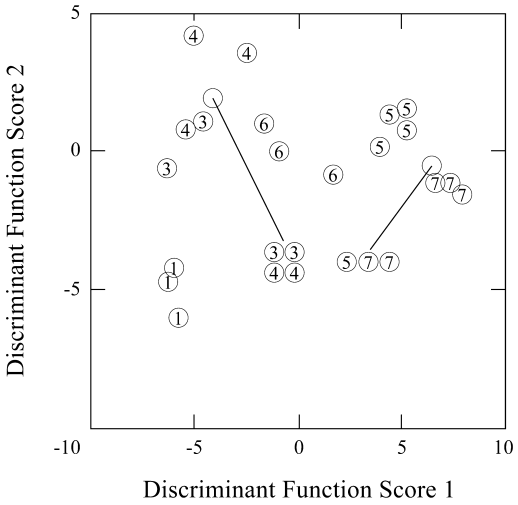

Figure 3. Discriminant function analysis of allele frequency data; numbers in circles correspond to locality designations in fig. 1.

Discussion

Among localities in Morocco, mtDNA *p*-distances (calculated as indicated in materials and methods, and fig. 2) range between 0.35 and 0.80 among northern (fig. 1: 5, 6, 7a) samples, and between 1.87 and 2.94 among southern (fig. 1: 7b, 8, 9) samples. The number of transitions within the three genes we considered was also considerably higher in southern samples, while only the number of transversions in ND4 appears higher (results, table 4). Nei's $\hat{D}$ values for allozyme comparisons between samples from northern Morocco range between 0.007 and 0.061, and graphical representation of these data suggests that localities 5 and 7 may be more closely related to each other than either is to locality 6 (tables 3 and 6, fig. 3).

When samples from northern Morocco are compared to those from southern Morocco,

p-distances vary from 2.43 to 3.19. Values for the number of transitions and transversions between northern and southern samples appear similar to those found in southern Morocco alone, suggesting that numbers of transitions and transversions in southern Moroccan samples are contributing heavily to this comparison. Comparisons between southern Spain and northern Morocco and between southern Spain and southern Morocco suggest that values both for p-distances and for numbers of transitions and transversions are similar between samples from southern Spain and from Morocco in general. Similar results are obtained for both p-distances and numbers of transitions and transversions between samples from northern Spain and samples from both northern and southern Morocco (results, table 4).

Within southern Spain (fig. 1: 2,4), mtDNA p-distances average 0.43 between samples. The numbers of transitions and transversions within ND2 and ND4 appear similar to those seen in samples from northern Morocco. When, however, samples from northern Spain (fig. 1: 1) are compared with samples from southern Spain (fig. 1: 2 and 4), the average p-distance for ND4 alone is 2.24 and the 95% confidence interval for the mean number of transitions is included within the confidence interval calculated for ND4 in a northern Morocco - southern Morocco comparison; only one transversion was identified in comparisons between samples from northern Spain and samples from southern Spain (results, table 4). Samples of *Psammodromus algirus* from north of Madrid (fig. 3: 1) and those from south of the Río Guadalquivir in Spain (fig. 3: 3 and 4) also exhibit allozyme differentiation. Nei's $\hat{D}$ values range between 0.098 and 0.105 between our samples, and graphical representation suggests *P. algirus* from northern Spain to be genetically distinct from those inhabiting southern Spain.

Within Morocco, p-distance and geographic distance appear linked; we cannot address this relationship within Spain. If we consider only African and Iberian localities likely to have been affected by the formation of the Strait of Gibraltar, this apparently-linked relationship between p-distance and geographic distance no longer holds. Nei's $\hat{D}$ values demonstrate significant correlations with geographic distance in both intracontinental and intercontinental comparisons, although it must be noted that the strength of the correlation is highest between localities presumably affected by the Strait's formation (see results).

Mitochondrial DNA p-distances between area samples inhabiting the north shore and south shore of the Strait of Gibraltar are substantially higher over shorter geographic distances than corresponding p-distances between area samples within Morocco (tables 4 and 5: below diagonal). Conversely, Nei's $\hat{D}$ values based on 35 presumptive gene loci present a somewhat more complex picture. Within Spain, two localities (fig. 1: 3 and 4) separated by approximately 22 km demonstrate a relatively low Nei's $\hat{D}$ value (0.013) between them while relationships among three localities in northern Morocco appear less obvious. Tanger and Chechaouèn (fig. 1: 5 and 7; fig. 3), separated by 90 km, appear more closely related, in a genetic sense (Nei's $\hat{D} = 0.007$), than do Tanger and Ksar-es-Seghir (fig. 1: 5 and 6; fig. 3), two localities separated by only 25 km (Nei's $\hat{D} = 0.026$). In the same sense, Ksar-es-Seghir and Chechaouèn, separated by 79 km, do not appear as closely related (Nei's $\hat{D} = 0.061$). When comparisons involving southern Spain are taken into consideration, samples from Benalup de Sidonia and Facinas (fig. 1: 3 and 4) appear somewhat more closely-associated with those from Ksar-es-Seghir (Nei's $\hat{D} = 0.038$ and 0.048, respectively) than with those from Tanger (Nei's $\hat{D} = 0.056$ and 0.065, respectively).

If ancestral *Psammodromus algirus* inhabited Iberia and North Africa while the Strait of Gibraltar was being formed and stabilized (Miocene-Pliocene), associated physiogeographic changes likely influenced their evolution. If sex-limited mitochondrial markers reflect deep phylogenetic history, the higher lev-

els of transitions, transversions, and *p*-distances found among southern Moroccan populations suggest that these lizards likely evolved in southern Morocco and expanded northward. If they had reached what is now southern Spain as events associated with the formation of the Strait of Gibraltar unfolded, Spanish and northern Moroccan representatives would have become dissociated from southern Moroccan stock.

As geologic change progressed (Benson et al., 1991; de Jong, 1998), and the region became less fragmented, bi-parentally inherited allozyme markers likely begin to reflect movement and assembly more accurately than does mtDNA. Populations inhabiting the area connecting Tanger and Chechaouèn probably shared an insular landmass as northern Morocco became more emergent, while populations inhabiting Ksar-es-Seghir were separated from those at Chechaouèn for a longer, albeit variable, time period. Concomitantly, Spanish populations inhabiting the areas around Benalup de Sidonia and Facinas were apparently more closely-associated over a longer time period with those from Ksar-es-Seghir than they were with those from Tanger. Additionally, southern Spanish representatives would have been temporally limited in their northward expansion by the (then) marine incursion (now) known as the Río Guadalquivir, and later variously affected by late Miocene-early Pleistocene changes in eastern Spain as the Betic Strait deteriorated and the Betic Cordillera developed (De Jong, 1998; García and Arsuaga, 2003).

Psammodromus algirus in the vicinity of the Strait of Gibraltar experienced geographic change similar to that experienced by *Podarcis erhardi*, for which Poulakakis et al. (2003) calculated the rate of sequence change in cytochrome *b* to be 1.45% to 1.59% per million years. If the rate of change in polymorphism is similar between *P. algirus* and *P. erhardi*, our estimates for average pairwise sequence polymorphism in cytochrome *b* (4.68-4.74%; results and table 4) for samples from northern and southern shores of the Strait (fig. 1: 2+4, 5, and 6) suggest these populations would last have been in complete reproductive contact between 2.98 and 3.23 million years ago. Further, southern Spanish populations (fig. 1: 2 + 4) would last have been in complete reproductive contact between 1.40 and 1.54 million years ago. While *p*-distance values we calculated for cytochrome *b* ranged between 0.18 and 3.15, the slope of the regression we used to estimate across-the-Strait estimates, and the correlation between calculated and estimated values were both significant ($P = 0.00$ and $P \ll 0.001$, respectively; see results). Our estimated *p*-distances are appropriate, therefore, in spite of the fact that they surpass calculated values by approximately 1.6%.

Higher levels of differentiation in mtDNA, coupled with higher levels of differentiation among presumptive gene loci (tables 4-6), across similar geographic distances indicate that *Psammodromus algirus* has a more extensive and complex history in Morocco than in Spain. Decidedly higher levels of average mitochondrial DNA differentiation, coupled with variable levels of differentiation among presumptive gene loci (tables 4-6), between area samples presumably separated as the Strait of Gibraltar formed indicates that this event did effect a barrier to gene exchange in this taxon. Spanish populations became isolated on insular areas between the Betic and Rif Straits, and possibly on the Iberian mainland while ancestral Moroccan populations likely shared these insular areas and were restricted to mainland Africa during formation of the Strait of Gibraltar.

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References

Arévalo, E., Davis, S.K., Sites, J.W., Jr. (1994): Mitochondrial DNA sequence divergence and phylogenetic relationships among eight races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology **43**: 387-418.

Ayala, F.J., Powell, J.R., Tracey, M.L., Mourão, C.A., Perez-Salas, S. (1972): Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics **70**: 113-139.

Benson, R.H., Rakic-El Beid, K., Bonaduce, G. (1991): An important current reversal (influx) in the Rifian Corridor (Morocco) at the Tortonian-Messinian boundary: the end of the Tethys Ocean. Paleoceanography **6**: 164-192.

Bonnet, E., Van de Peer, Y. (2001): zt: a software tool for simple and partial Mantel tests. (eric.bonnet@gengenp.rug.ac.be)

Burbrink, F.T., Lawson, R., Slowinski, J.B. (2000): MtDNA phylogeography of the North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution **54**: 2107-2118.

Busack, S.D. (1986): Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. National Geographic Research **2**: 17-36.

Busack, S.D., Jaksić, F.M. (1982): Ecological and historical correlates of Iberian herpetofaunal diversity: an analysis at regional and local levels. Journal of Biogeography **9**: 289-302.

Busack, S.D., Lawson, R., Arjo, W.M. (2005): Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex. Amphibia-Reptilia **26**, 235-256.

Carranza, S., Arnold, E.N. (2004): History of West Mediterranean newts, *Pleurodeles* (Amphibia:Salamandridae), inferred from old and recent DNA sequences. Systematics and Biodiversity **1**: 327-337.

Carranza, S., Arnold, E.N., Wade, E., Fahd, S. (2004): Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populkations arrived recently from Northwest Africa. Molecular Phylogenetics and Evolution **33**: 523-532.

Civantos, E., Forsman, A. (2000): Determinants of survival in juvenile *Psammodromus algirus* lizards. Oecologia **124**: 64-72.

de Jong. (1998): In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. Biological Journal of the Linnean Society **65**: 99-164.

de Queiroz, A., Lawson, R., Lemos-Espinal, J.A. (2002): Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? Molecular Phylogenetics and Evolution **22**: 315-329.

Diaz, J.A., Carbonell, R., Virgos, E., Santos, T., Telleria, J.L. (2000): Effects of forest fragmentation on the distribution of the lizard *Psammodromus algirus*. Animal Conservation **3**: 235-240.

Feldman, C.R., Parham, J.F. (2004): Molecular systematics of Old World Stripe-necked turtles (Testudines: *Mauremys*). Asiatic Herpetological Research **10**: 28-37.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

García, N., Arsuaga, J.L. (2003): Last glaciation cold-adapted faunas in the Iberian Peninsula. Deinsea **9**: 159-169.

Guillaume, C.P. (1197): *Psammodromus algirus*. In: Atlas of Amphibians and reptiles in Europe, p. 302-303. Gasc, J.-P., Cabela, A., Crnobrnja-Isailovic, J., Dolman, D., Grossenbacher, K., Haffner, P., Lescure, J., Martens, H., Martínez Rica, J.P., Maurin, H., Oliveira, M.E., Sofianidou, T.S., Veith, M., Zuiderwijk, A., Eds, Paris, Societas Europaea Herpetologica and Muséum National d'Histoire Naturelle.

Harris, D.J., Batista, V., Carretero, M.A. (2004a): Assessment of genetic diversity within *Acanthodactylus erythrurus* (Reptilia: Lacertidae) in Morocco and the Iberian Peninsula using mitochondrial DNA sequence data. Amphibia-Reptilia **25**: 227-232.

Harris, D.J., Batista, V., Carretero, M.A., Ferrand, N. (2004b): Genetic variation in *Tarentola mauritanica* (Reptilia: Gekkonidae) across the Strait of Gibraltar derived from mitochondrial and unclear DNA sequences. Amphibia-Reptilia **25**: 451-459.

Harris, D.J., Batista, V., Lymberakis, P., Carretero, M.A. (2004c): Complex estimates of evolutionary relationships in *Tarentola mauritanica* (Reptilia: Gekkonidae) derived from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution **30**: 855-859.

Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. Journal of Biogeography **29**: 1257-1262.

Harris, H., Hopkinson, D.A. (1976): Handbook of enzyme electrophoresis in human genetics. Amsterdam, North Holland Publishing Company.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences **86**: 6196-6200.

Kumazawa, Y., Ota, H., Nishida, M., Ozawa, T. (1996): Gene arrangements in snake mitochondrial genomes: highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Molecular Biology and Evolution **13**: 1242-1254.

Kumazawa, Y., Nishida, M. (1999): Complete mitochondrial sequences of the green turtle and blue-tailed mole skink: Statistical evidence for archosaurian affinity of turtles. Molecular Biology and Evolution **16**: 284-292.

Martínez-Solano, Í. (2004): Phylogeography of Iberian *Discoglossus* (Anura:Discoglossidae). Journal of Zoological Systematics and Evolutionary Research **42**: 298-305.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583-590.

Paulo, O.S., Pinto, I., Bruford, M.W., Jordan, W.C., Nichols, R.A. (2002): The double origin of Iberian peninsular chameleons. Biological Journal of the Linnean Society **75**: 1-7.

Pérez-Mellado, V. (1998): *Psammodromus algirus* (Linnaeus, 1758). In: Reptiles, p. 307-318. Salvador, A. (Coordinator), 1998. Fauna Iberica, vol. 10. Ramos, M.A. et al. (Eds). Madrid, Museo Nacional de Ciencias Naturales.

Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E. (2003): Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). Molecular Phylogenetics and Evolution **28**: 38-46.

Schleich, H.H., Kästle, W., Kabisch, K. (1996): Amphibians and Reptiles of North Africa. Koenigstein (Germany), Koeltz Scientific Publishers.

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E., Gentry, J.B. (1971): Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI, University of Texas Publications **7103**: 49-90.

Sokal, R.R., Rohlf, F.J. (1998): Biometry. New York, W. H. Freeman and Company.

Systat Software, Inc. (2004): SYSTAT 11.0. Point Richmond, California 94804-2028, U.S.A. (501 Canal Blvd., Suite C; http://www.systat.com).

Swofford, D.L. (2000): PAUP*: phylogenetic analysis using parsimony. Version 4.0b4a. Sunderland, Massachusetts, U.S.A., Sinauer Associates, Inc.

Veith, M., Mayer, C., Samraoui, B., Donaire Barroso, D., Bogaerts, S. (2004): From Europe to Africa and vice versa: evidence for multiple intercontinental dispersal in ribbed salamanders (Genus *Pleurodeles*). Journal of Biogeography **31**: 159-171.

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Appendix: Specimens examined

(Geographic coordinates from Official Standard Names Gazetteers for Morocco [1970] and Spain [1961] published by the United States Board on Geographic Names, Department of the Interior, Washington, D.C.)

DNA Analysis. *Podarcis muralis*: SPAIN (Huesca): Benasque (42°36′N, 0°32′W), Baños de, MNCN 23636. *Psammodromus algirus*: MOROCCO (Marrakech): Oukaïmedèn (31°13′N, 7°52′W), MVZ 178364; (Meknès): Âïn Leuh (33°17′N, 5°23′W), MVZ 178365; (Tanger): Cap Spartel (35°48′N, 5°56′W), MVZ 178375; Tanger (35°48′N, 5°48′W), MVZ 186209; (Tétouan): Chechaouèn (35°10′N, 5°16′W), MVZ 178380-178381; Ksar-es-Seghir (35°51′N, 5°34′W), MVZ 178390. SPAIN (Cádiz): vicinity of Facinas (36°08′N, 5°42′W), MNCN 11940-11943; (Madrid): Manzanares el Real (40°44′N, 3°52′W), MNCN 11957 and 11959; (Málaga): Río Hozgarganta at km 68 on roadway C-3331, MNCN 11956.

Allozyme Analysis. *Psammodromus algirus*: MOROCCO (Tanger): Cap Spartel, MVZ 178371-178374; Tanger, MVZ 178369; (Tétouan): Chechaouèn, MVZ 178382-178386; Ksar-es-Seghir, MVZ 178390-178392. SPAIN (Cádiz): vicinity of Benalup de Sidonia (36°20′N, 5°49′W), MNCN 11951-11953 and Busack field series SDB 1653 (voucher apparently lost, population represented by MNCN 11952 and 11953); vicinity of Facinas, MNCN 11940-11944; (Madrid): Manzanares el Real, MNCN 11957-11959.