A new perspective on the zoogeography of the sibling mouse-eared bat species *Myotis myotis* and *Myotis blythii*: morphological, genetical and ecological evidence

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The actual geographic distribution of the two sibling mouse-eared bat species *Myotis myotis* and *Myotis blythii*, which occur widely sympatrically in the western Palaearctic region, remains largely controversial. This concerns particularly the specific attribution of marginal populations from the Mediterranean islands and from adjacent areas of North Africa and Asia, which are morphologically intermediate between continental *M. myotis* and *M. blythii* from Europe. This study attempts to clarify this question by using four different approaches: cranial morphology, external morphology, genetics and trophic ecology. The three latter methods show unambiguously that North Africa, Malta, Sardinia and Corsica are presently inhabited by monospecific populations of *M. myotis*. In contrast, cranial morphometrics do not yield conclusive results. These results contradict all recent studies, which attribute North African and Maltese mouse-eared bats to *M. blythii* and consider that Sardinia and Corsica harbour sympatric populations of the two species. As concerns south-eastern populations, doubts are also expressed about the attribution of the subspecific taxon *omari* which may actually refer to *M. myotis* instead of *M. blythii*. Protein electrophoresis is presently the only absolute method available for determining *M. myotis* and *M. blythii* throughout their distribution ranges. However, species identification may be approached by relying on less sophisticated morphometrical methods as presented in this study. Species-specific habitat specializations are probably responsible for the differences observed between the geographic distributions of *M. myotis* and *M. blythii*, as they provide a logical groundwork for a coherent model of speciation for these two bat species.

Introduction

A vast literature [see the review by Arlettaz (1995)] has been published on the systematics and biogeography of the two cryptic bat species *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii* (Tomes, 1857) which occur sympatrically in the western Palaearctic region, but the identity of many populations remains largely controversial, especially in Mediterranean islands, North Africa and the Near East. The genetic validity of two distinct species has been appropriately documented only in continental Europe (Ruedi, Arlettaz & Maddalena, 1990), where *M. myotis* and *M. blythii* show a close morphological resemblance and often form mixed roosting groups but do not interbreed (Ruedi *et al.*, 1990; Arlettaz, Ruedi & Hausser, 1991). No similar data are available for other geographic regions. Because morphological characters such as overall size may be both adaptive and subject to character displacement, taxonomic reviews by previous authors are of limited value to reconstruct the zoogeographical history of these two species (Ellerman & Morrison-Scott, 1965; Findley, 1971;

Strelkov, 1972; Felten, Spitzenberger & Storch, 1977; Bogan *et al.*, 1978; Corbet, 1978; Koopman, 1993). Thus, a basic knowledge of the distributions of both mouse-eared bat species remains unclear. The present study attempts to clarify the zoogeography of *M. myotis* and *M. blythii* in the western part of their range by considering cranial morphology, external morphology, genetics and ecology.

Material and methods

Morphometrics

Cranial morphology

Seven different skull measurements were taken on 56 specimens (31 *M. myotis* and 25 *M. blythii*) collected from 12 regions in Morocco, Spain, Sardinia, Corsica, Switzerland, Turkey and Kirghizstan (Table I). These variables are greatest skull length (GL, measured from the second incisive), interorbital constriction (IC), braincase width (BC), zygomatic breadth (ZB), inter-canines width (CC), inter-molars width (MM) and maxillary tooth row (canines to molars, CM^3). These measurements are illustrated in Arlettaz (1995). To minimize the impact on natural populations, only 2–7 individuals were collected from each region. Because skull size is correlated with body size, the smallest and the largest individuals were selected among the bats captured in each region. Skull size was not representative of a population as a whole, but was most likely to include individuals of the 2 species in sympatric populations. Further material previously collected was also measured (Morocco and Spain, Estación Biológica Doñana, Sevilla, Collection C. Ibañez, $n = 20$; Institute of Zoology and Animal Ecology, Lausanne, Collection M. Ruedi, $n = 3$). For all specimens tissues were also available for genetic analyses.

External morphology

Forearm length and ear length were measured on all living individuals ($n = 503$ *M. myotis* and 426 *M. blythii*) captured in 11 populations; discriminant scores were computed according to the method outlined in Arlettaz, Ruedi & Hausser (1991). In central Europe, this technique allowed a good separation of the 2 species, with a probability of correct classification of over 99%. The presence of a white spot of hair on the head, typical of *M. blythii*, was also checked (Arlettaz *et al.*, 1991).

Genetics

All individuals collected were identified by an allozyme analysis of tissue samples according to the methods outlined in Ruedi *et al.* (1990). Two loci discriminant among *M. myotis* and *M. blythii* in western and central Europe were used to identify each individual: GOT-1 (Enzyme Commission number 2.6.1.1) and MPI (EC 5.3.1.8). We assessed genetic variability between various samples with 7 additional loci. These are ADA (EC 3.5.4.4) , G-6-PD (EC 1.1.1.49), GOT-2 (EC 2.6.1.1), ME-1 and ME-2 (EC 1.1.1.40), 6-PGD (EC 1.1.1.44) and GPI (EC 5.3.1.9). Buffer solutions and electrophoretic conditions are described in Ruedi *et al.* (1990), except for ADA which was run on a Phosphate buffer (pH 6.7) for 15 hours at 45 V/cm and stained according to Harris & Hopkinson (1976). Allele frequencies for each population were computed and transformed into unbiased genetic distances (Nei, 1978). The genetic relationships between the populations were represented on a UPGMA phenogram (Sneath & Sokal, 1973). Precise location and access number to reference samples are available upon request to the senior author.

Ecology

The diet of both species was studied by faecal analysis (Kunz & Whitaker, 1983; Dickmann & Huang, 1988) using individual faecal samples collected from live-trapped individuals in 9 populations: North and South

TABLE I

Origin of the material investigated for skull morphology and genetics, external morphology, and faecal analysis

Locality	Number of collection sites	Approximate coordinates	Individuals collected for skulls and tissues			Individuals measured for external morphology		Faecal samples	
			M. myotis	M. blythii	Collection[1]	M. myotis	M. blythii	M. myotis	M. blythii
Switzerland									
Valais (Fully, Raron, Naters)	4	46°15'N, 07°30'E	5	5	IZEA	105	146	152	169
Switzerland NE[2]	10	47°20'N, 09°00'E				67	28	19	15
Portugal (Lousal, Querença)	2	37°40'N, 08°40'W				36	18	13	
Spain									
Andalusia (Jerez, Cadiz, Tarifa, Malaga)	4	36°30'N, 06°30'W	8	5	EBD, IZEA	12	22		
Rioja	1	42°30'N, 02°30'W	2		EBD				
Cataluña (Barcelona)	1	41°30'N, 02°00'E	2	1	IZEA				
Levante (Valencia)	1	39°30'N, 00°30'W		3	EBD				
Morocco									
Rif (Tanger, Tetouan)	2	35°50'N, 05°30'W	6		EBD, IZEA	10			
Middle Atlas (Berkane, Taza Merhraoua, El Hajeb)	4	34°10'N, 03°00'W / 33°50'N, 04°00'W	4		IZEA	69		21	
Rabat	1	34°00'N, 07°00'W				13			
Sahara (Tazouguerte)	1	32°00'N, 03°20'W	1		IZEA	20			
Malta	1	35°50'N, 14°30'E						2	2
Sardinia (Mt. Maiore, Borutta, Pozzomaggiore, Ulàssai)	4	40°30'N, 09°00'E	3		IZEA	100		8	
Corsica (Oletta, Castifao)	2	42°30'N, 09°15'E	2		IZEA	71		13	
Turkey (Döngel Magarasi)	1	38°00'N, 36°30'E	2		IZEA				
Kirghizstan SW (Os, Kyzyl-Kija)	7	40°20'N, 72°30'E		7	IZEA		212		58
Total	46		35	21		503	426	230	242

[1] IZEA = Institute of Zoology and Animal Ecology, University of Lausanne, Switzerland; EBD = Estación Biológica Doñana, Sevilla, Spain

[2] For more details about the location of these 10 localities, see Arlettaz et al. (1994)

Morocco, Portugal, Malta, Sardinia, Corsica, NE and SW Switzerland, and Kirghizstan (for sample sizes, see Table I). The percentage volume of ground-dwelling and grass-dwelling prey categories, typical of *M. myotis* and *M. blythii*, respectively, was assessed [see Arlettaz & Perrin (1995) for more details]. In the Swiss sympatric populations, these 2 main prey categories are, species-specifically, the dominant prey groups throughout the season (Arlettaz, 1995).

Statistics

Principal component (PCA) and discriminant analyses were performed on morphological variables and all analyses were computed using the Systat package for the Macintosh (Wilkinson, Hill & Vang, 1992).

Results

Morphometrics

Cranial morphology

A principal component analysis (PCA) performed on the covariance matrix showed that the first factor (PC1) accounted for 89% of the overall variance, whereas the second factor (PC2) accounted for only 4% (Fig. 1). PC1 was highly correlated with variables expressing skull length, tooth row length and skull width (GL, r = 0.989; CM^3, r = 0.965; ZB, r = 0.937). PC2 was most correlated with maxillary width (CC, r = 0.415; MM, r = 0.351) (Table II). A plot showing the relationship between these first two components (Fig. 1) clustered the populations of mainland Europe in two distinct groups, comprising, respectively, the smaller and the larger individuals. Individuals from Mediterranean islands and North Africa (Morocco) grouped together in an intermediate cluster, whereas the two Turkish specimens chosen for their extreme phenotypes fell in the middle and right clusters. Kirghiz mouse-eared bats were clearly the smallest individuals. A second PCA performed on the same variables adjusted for size (i.e. divided by GL and log-normalized) did not yield a clearer grouping.

External morphology

The frequency distribution of the discriminant scores obtained for the various populations showed a clear bimodality for Switzerland and Spain, but not for the Portuguese samples which clustered near zero. The distribution of scores for Morocco, Sardinia and Corsica was unimodal and coincided more closely with the Swiss *M. myotis*, whereas the distribution of Kirghiz individuals was unimodal and coincided with the Swiss *M. blythii* (Fig. 2). Only some of the individuals from Switzerland, Spain and Portugal had a spot of white hairs on the head, a feature characteristic of *M. blythii* (Arlettaz *et al.*, 1991). By contrast, nearly all (i.e. > 95%) individuals from Kirghizstan exhibited this character, whereas no individual from Morocco, Sardinia and Corsica did so. Thus, both the frequency distribution of discriminant scores and the presence/absence of the white spot suggest: 1) the occurrence of sympatric populations of the two species in the three countries of continental Europe; 2) the existence of monospecific populations of *M. blythii* in Kirghizstan; and 3) the occurrence of monospecific populations of *M. myotis* in Morocco, Sardinia and Corsica.

Genetics

Among the nine studied loci, four were monomorphic across all individuals (GOT-2, ME-1 and 2, G-6-PD). Of the polymorphic ones, MPI could not be reliably scored in all populations and was
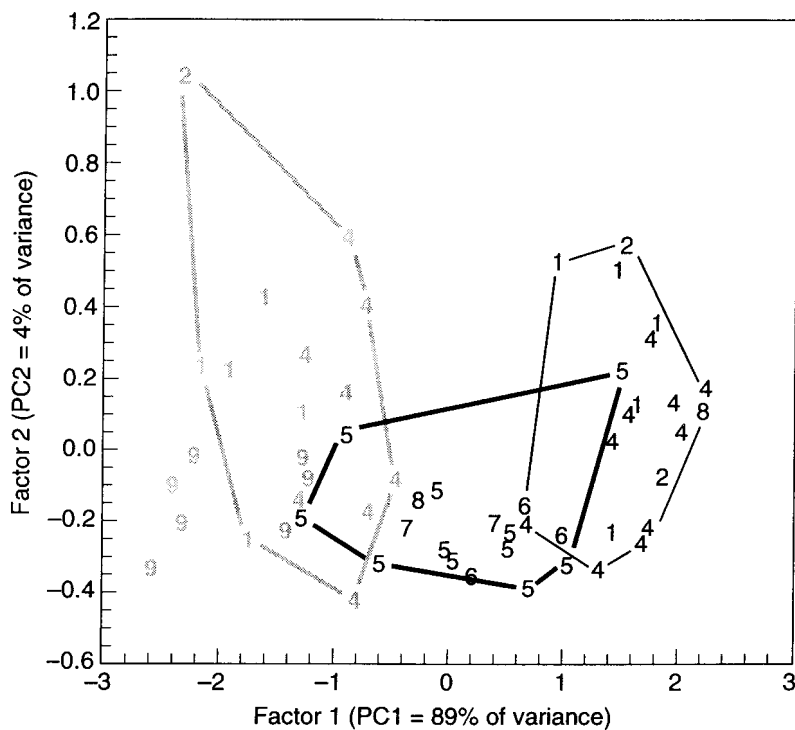
FIG. 1. Relationships between Factor 1 and Factor 2 (Principal component analysis, PCA) for 56 mouse-eared bat skulls from various origins. Individuals from continental Europe (shown by symbols 1, 2, 4) clustered in two distinct groups [smaller individuals (on the left) and larger ones (on the right)], whereas mouse-eared bat populations from the Mediterranean islands and Morocco appeared intermediate (middle cluster). Note that the two Turkish specimens (8) were split between the middle and right clusters, and that Kirghiz individuals (9) were the smallest specimens. 1 = Switzerland; 2 = Cataluña; 4 = Spain (except Cataluña); 5 = Morocco; 6 = Sardinia; 7 = Corsica; 8 = Turkey; 9 = Kirghizstan.

TABLE II

Correlation matrix showing the relationships between cranial variables and factors 1 and 2 (PC1 and PC2) obtained from principal component analysis. For variables and PC1, all correlation coefficients were highly significant (P < 0.007). For variable abbreviations, see **Material and methods**

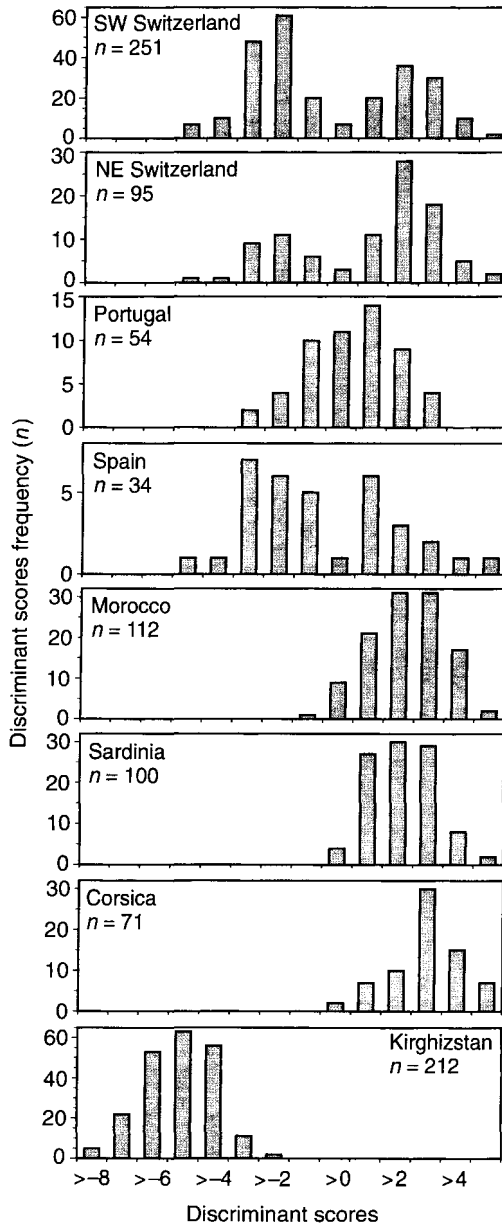| Variable	GL	ZB	BC	IC	CC	MM	CM^3
ZB	0.900						
BC	0.681	0.742					
IC	0.385	0.427	0.566				
CC	0.751	0.790	0.611	0.439			
MM	0.823	0.811	0.615	0.364	0.842		
CM^3	0.960	0.852	0.592	0.356	0.752	0.848	
PC1	0.989	0.937	0.713	0.418	0.813	0.881	0.965
PC2	−0.137	0.162	0.261	0.265	0.415	0.351	−0.111

Fig. 2. Frequency distribution of the scores obtained from a discriminant function including two external characters (forearm and ear length) and applied to different mouse-eared bat populations. This equation allowed a correct classification of more than 99% Swiss individuals whose identity was biochemically assessed (Arlettaz *et al.*, 1991). Distributions are bimodal for Switzerland and Spain, but unimodal for Morocco, Sardinia, Corsica and Kirghizstan; distribution also appears unimodal for Portugal but specimens were grouped together near zero (for more details about localities, see Table I).

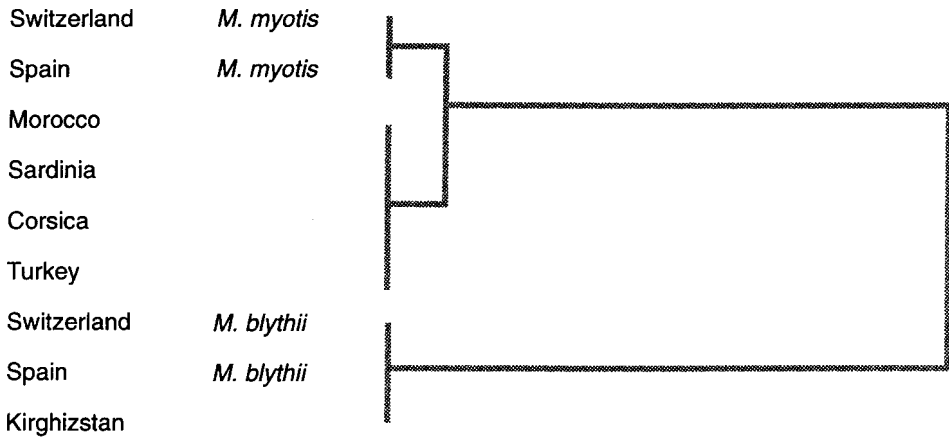|		
Switzerland	*M. myotis*	
Spain	*M. myotis*	
Morocco		
Sardinia		
Corsica		
Turkey		
Switzerland	*M. blythii*	
Spain	*M. blythii*	
Kirghizstan		

FIG. 3. UPGMA phenogram showing the genetic relationships between the different mouse-eared bat populations investigated by protein electrophoresis (see Table IV). Populations from Mediterranean islands, North Africa and Turkey clustered with continental *M. myotis*, whereas Kirghiz individuals were grouped within the *M. blythii*'s cluster.

therefore excluded from subsequent analyses. Allele frequencies of the four variable loci are presented in Table III. GOT-1 and ADA proved to be fixed for distinct alleles in the parental species in Switzerland, Spain and Kirghizstan. They were thus used to diagnose other populations of uncertain status. Under this criteria, samples from Morocco, Sardinia, Corsica and Turkey all represented *M. myotis*. Although not fully discriminant, 6-PGD also supports the same identification, as the most common allele (+100) found in continental *M. myotis* is also shared by these unidentified populations; *M. blythii* from Switzerland, Spain or Kirghizstan are all fixed for another allele (+115). Table IV presents the matrix of genetic distances (Nei, 1978) between all pairs of populations. Because of the small number of loci examined, genetic distances should be used for relative comparisons only. However, the UPGMA dendrogram based on Nei's genetic distances showed a sharp dichotomy: the distance between the two main clusters is much greater than the second greatest distance, which separated the continental *M. myotis* from the Mediterranean ones (Fig. 3). Indeed, populations from Mediterranean islands, North Africa and Turkey clustered with the Swiss and Iberian *M. myotis*, whereas Kirghiz mouse-eared bats grouped together with Swiss and Iberian *M. blythii*.

Trophic ecology

The proportions of ground- vs. grass-dwelling prey in the diets of mouse-eared bats from Morocco, Malta, Sardinia and Corsica were similar to the proportions found in Swiss and Portuguese *M. myotis*, whereas the food of Kirghiz mouse-eared bats most resembled the diet of *M. blythii* from continental Europe (Fig. 4).

Discussion

A critical zoogeographical review

The study of external morphology, genetics and dietary ecology showed that monospecific populations of *M. myotis* inhabit Morocco, Sardinia and Corsica. By contrast, cranial morphometrics

TABLE III

Allelic frequencies for the four polymorphic protein loci revealed from mouse-eared bats originating from various populations. Allele designations according to Ruedi et al. (1990)

Locus	Allele	Switzerland		Spain		Morocco	Sardinia	Corsica	Turkey	Kirghizstan
		M. myotis 5	*M. blythii* 5	*M. myotis* 12	*M. blythii* 9	11	3	2	2	7
6-PGD	+115	0.25	1.00	0.10	1.00	0.05	—	—	—	1.00
	+100	0.75	—	0.90	—	0.95	1.00	1.00	1.00	—
ADA	+100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPI	+90	—	1.00	—	1.00	—	1.00	1.00	1.00	1.00
	−60	0.47	—	0.35	—	0.95	—	—	—	—
	−100	0.53	—	0.65	—	0.05	—	—	—	—
GOT-1	−15	—	1.00	—	1.00	—	—	—	—	1.00
	−20	—	—	—	—	—	—	—	0.25	—
	−100	1.00	—	1.00	—	1.00	1.00	1.00	0.75	—

TABLE IV

Nei's genetic distance between the different populations of mouse-eared bats investigated by enzyme electrophoresis. Because only the four polymorphic loci were computed, genetic distances should be used for relative comparisons only

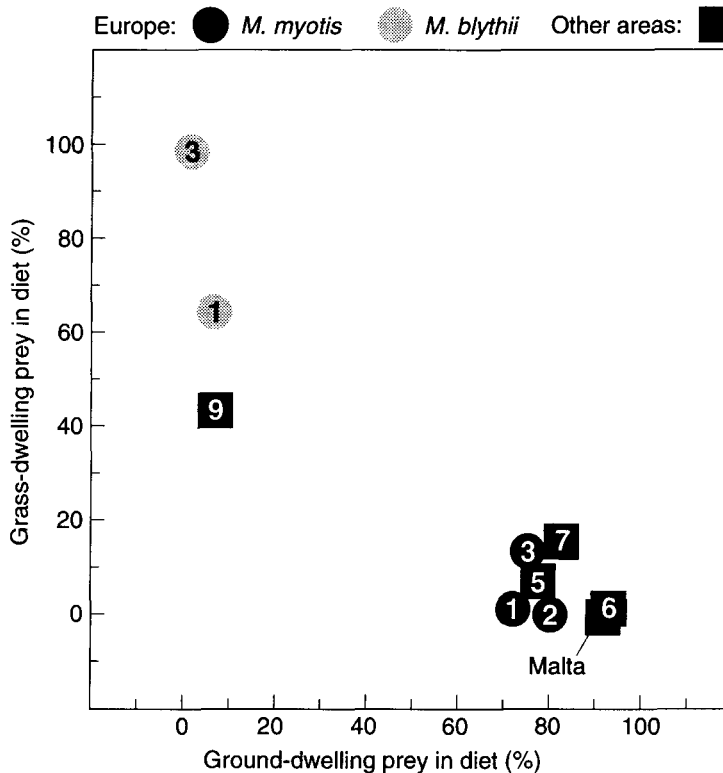|		Switzerland		Spain					
		myotis	*blythii*	*myotis*	*blythii*	Morocco	Sardinia	Corsica	Turkey
Switzerland	*myotis*	—							
	blythii	1.582	—						
Spain	*myotis*	0.000	2.093	—					
	blythii	1.582	0.000	2.093	—				
Morocco		0.065	1.361	0.098	1.361	—			
Sardinia		0.084	1.386	0.116	1.386	0.000	—		
Corsica		0.084	1.386	0.116	1.386	0.000	0.000	—	
Turkey		0.098	1.320	0.129	1.320	0.000	0.000	0.000	—
Kirghizstan		1.582	0.000	2.093	0.000	1.361	1.386	1.386	1.320

FIG. 4. Proportion of ground- and grass-dwelling prey in the diet of the different populations of mouse-eared bats. The diets of populations from North Africa and Mediterranean islands were close to the ones of continental *M. myotis* from Europe, whereas the diet of Kirghiz mouse-eared bats clustered with the diets of Swiss and Portuguese *M. blythii*. 1 = SW Switzerland; 2 = NE Switzerland; 3 = Portugal; 5 = Morocco; 6 = Sardinia; 7 = Corsica; 9 = Kirghizstan. Malta is also indicated.

did not yield conclusive results. The occurrence of only one mouse-eared bat species in North Africa (Morocco, Algeria, Tunisia and Libya) has been recognized by most authors (e.g. Ellerman & Morrison-Scott, 1965; Aellen & Strinati, 1970; Strelkov, 1972; Felten *et al.*, 1977; Bogan *et al.*, 1978; Hanak & Elgadi, 1984; Aulagnier & Thévenot, 1986; Horacek & Gaisler, 1986). However, most recent studies have attributed the North African mouse-eared bats to *M. blythii* (for an historical review about the specific attribution of North African mouse-eared bats, see Felten *et al.*, 1977: 38). Two investigators have expressed doubts about this specific identity. Strinati (1951) first referred all Moroccan specimens to *M. myotis*, but published a corrective note in 1953; Gaisler (1983) was surprised by the ear length of the specimens he collected in Algeria: ''Remarquons que la longueur de l'oreille est nettement plus grande chez les exemplaires de notre collection par comparaison avec celle des exemplaires de *M. b. oxygnathus* en Europe''. Subspecifically, North African mouse-eared bats were referred to *M. blythii oxygnathus* by former authors, and to *M. blythii omari* by Strelkov (1972). Felten *et al.* (1977) even proposed a new subspecies for North Africa, *M. blythii punicus*.

For Sardinia, Corsica and Malta, the taxonomy is also confused. Felten *et al.* (1977) referred Corsican, Sardinian and Maltese mouse-eared bats to *M. blythii punicus* instead of *M. b. omari*. In Sardinia, Frick & Felten (1952) and Miller (1966) only reported the presence of *M. myotis*. Lanza [and not ''Motta'' as written by Strelkov (1972); M. Mucceda, pers. comm.] classified Sardinian mouse-eared bats in the group *intermedia*, whereas Strelkov (1972) attributed most Sardinian mouse-eared bats to *M. blythii omari*, although he did not deny the possible presence of *M. myotis* on that island. Kahmann & Goerner (1956) identified all mouse-eared bats found on Corsica as *M. myotis* and attributed smaller specimens found in owl pellets to immature *M. myotis*. Beaucournu[1] (1965) reported the presence of a single *M. blythii* within a cluster of *M. myotis*, and Beaucournu, Launay & Noblet (1983) again referred to captures of the lesser species in Corsica. Menu & Popelard (1987) and Courtois, Faggio & Salotti (1992) suggested that specimens from Corsica may be morphologically closer to continental *M. myotis* than to mainland *M. blythii*. Specimens from Malta have been attributed to *M. blythii omari* (e.g. Strelkov, 1972) or to *M. blythii punicus* (Borg *et al.*, 1990). Strelkov (1972) considered that there was no substantiated reason to believe that *M. myotis* also occurred on that island. However, there is now dietary evidence (this study) indicating that Maltese mouse-eared bats may actually belong to *M. myotis*.

Because of their strong morphological similarity with Corsican, Sardinian and Maltese mouse-eared bats, specimens from Crete and Cyprus have been attributed to *M. blythii omari* (Strelkov, 1972; Spitzenberger, 1979; Iliopoulou-Georgudaki, 1979, 1984). Although we did not collect material from those islands, we would not be surprised that these two eastern Mediterranean islands also shelter populations of *M. myotis* and not of *M. blythii*. Incidentally, the distinction of the subspecies *M. blythii lesviacus* on Lesbos (Greece) seems hardly justifiable (Iliopoulou-Georgudaki & Giagia, 1984) until we know more about the specific identity of populations in that part of the Mediterranean.

As concerns Asian populations, it is generally agreed that *M. myotis* and *M. blythii* occur sympatrically in the Near East and Asia Minor, and that regions to the east of Kurdistan are occupied by *M. blythii* only (Harrison & Lewis, 1961; Strelkov, 1972; Felten *et al.*, 1977; Deblase, 1980; Helversen, 1989; Albayrak, 1990; Harrison & Bates, 1991). The smaller mouse-eared bats occurring in Arabia (*sensu lato*) have been referred to *M. blythii omari* [or to its synonym *M. b. risorius*, Harrison & Lewis (1961)] by most of these authors, although older reviews classified the eastern *omari* in

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[1]Note that in the same paper, Beaucournu (1965) attributed some specimens from north-eastern France (Anjou and Touraine) to *M. blythii*. However, a comparison of his measurements with our data from continental Europe suggests that these specimens belong to *M. myotis*

M. myotis (Thomas, 1905; Cheesman, 1921; Ellerman & Morrison-Scott, 1965). A comparison of the measurements of 36 skulls from Iran listed by Deblase (1980) with the measurements obtained in the present study suggests that pure populations of *M. myotis* may well occur in that region. Besides, Strelkov (1972) also noticed that "the size of Asian *M. b. omari* is very akin to *M. myotis*". Our material from this geographic area consists of only two specimens, one phenotypically large and the other small. The skulls of these two individuals are situated at both extremes of the overall skull variation in *M. myotis* (Fig. 1), but are unambiguously identified as *M. myotis* by the genetic analysis (Table III and Fig. 3). The smaller individual appears in the intermediate group, along with western Mediterranean and North African mouse-eared bats (*omari* and *punicus*); the larger one is situated at the outermost edge of the cluster grouping larger individuals from continental Europe (*myotis*) and could be referred to the larger subspecies *macrocephalicus* described by Harrison & Lewis (1961) from Palestine. Obviously, in the absence of more details on size variation within Turkish populations, it is presently impossible to attribute these mouse-eared bats to any subspecific name within *M. myotis*. However, the presence of both small and large animals identified as *M. myotis* in Turkey raises doubts about the specific affiliation of the eastern populations referred to the taxon *omari* (Transcaucasia, Near East, Arabia including Iran, and western Turkestan). In our opinion, it may represent *M. myotis* instead of *M. blythii*. Further genetic studies are needed in this area to solve this problem. Presently, it is impossible to draw a limit of *M. myotis*' range in the Middle East, while it seems clear that *M. blythii* extends further east to India and Mongolia (Fig. 5).

How to identify mouse-eared bats?

Allelic mobility at two allozyme loci (GOT-1 and ADA) provides the only absolute criteria which allow an unambiguous identification of single specimens (Table III). However, this diagnosis requires fresh material (either tissue or blood sample, see Ruedi *et al.*, 1990) and an appropriate laboratory setting that is not accessible to most bat workers. Species identification may be possible using less sophisticated morphometric methods (Table V; Figs 6 & 7). In this case, relatively large samples are still needed in order to evaluate individual variation within populations (see Fig. 2), and to differentiate the two sibling species accurately. Discriminant functions may be used for the identification of both skulls and live individuals. Calculated on the whole skull sample (canonical correlation coefficient, r = 0.834), a proposed discriminant function for species attribution is: $Z = 4.231 \times GL + 0.115 \times ZB + 1.682 \times CM^3 - 110.987$; if $Z > 0$, then *M. myotis*; if $Z < 0$, then *M. blythii*; this function has a probability of correct classification of 94%. As concerns external characters, $Z = 0.433 \times$ forearm length $+ 3.709 \times$ ear length $- 114.887$ (canonical correlation coefficient, r = 0.894); if $Z > 0$, then *M. myotis*, if $Z < 0$, then *M. blythii*; probability of correct classification is 98%. Because *M. myotis* and *M. blythii* seem to have a fairly specialized trophic ecology (Fig. 4; Arlettaz, 1995; Arlettaz & Perrin, 1995), faecal analysis may also provide a useful guide for species identification, as long as individual faecal samples may be collected.

Character displacement and niche shift

The study of external morphology showed that the distributions of discriminant scores did not vary between sympatric and allopatric populations to the extent predicted by the model of character displacement (Grant, 1972). Similarly, there was no apparent difference in the dominant prey groups (ground- versus grass-dwelling arthropods) of either species under sympatric versus allopatric conditions. This suggests that *M. myotis* and *M. blythii* have probably evolved species-specific
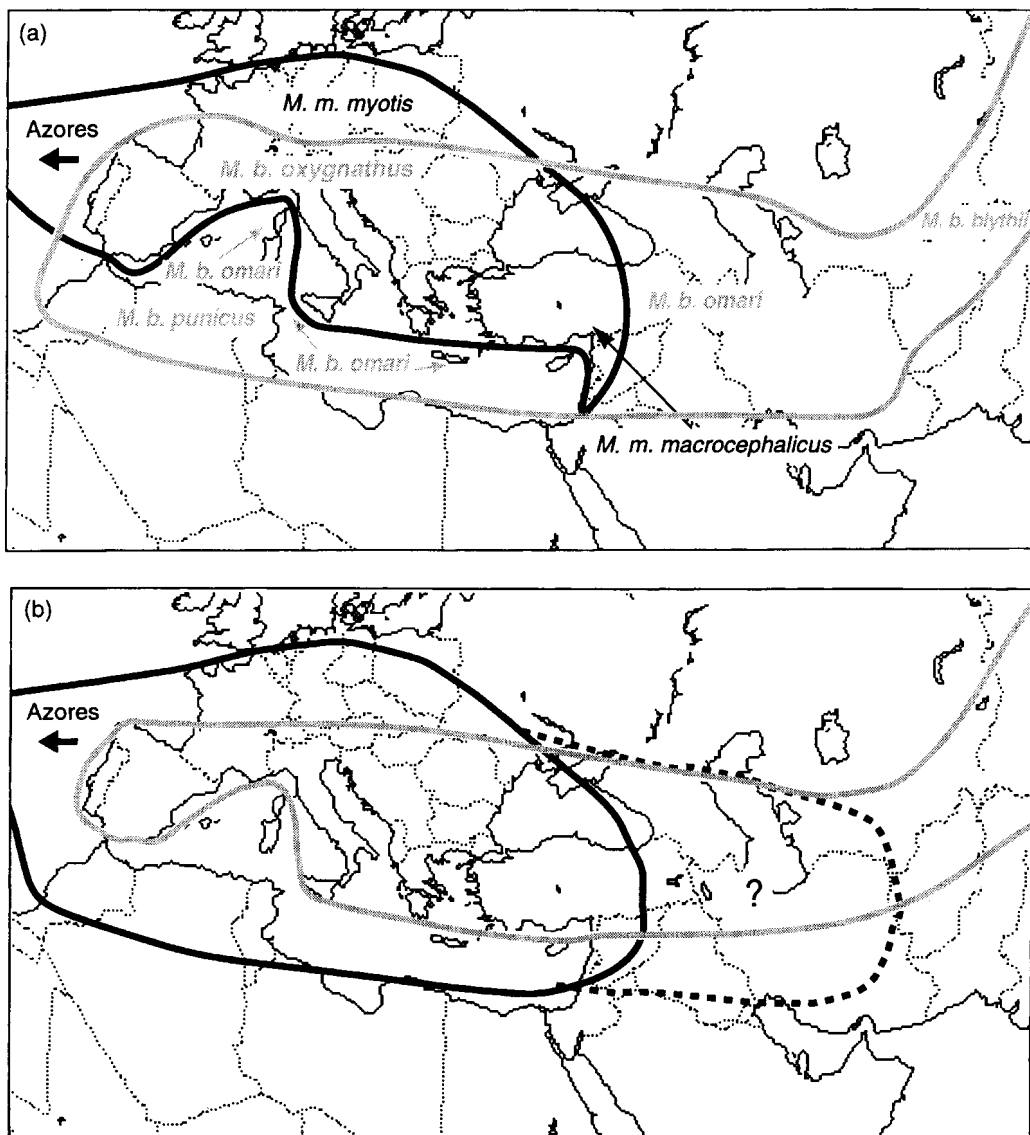
FIG. 5. (a) The prevailing view about the geographic distribution of *M. myotis* (black line) and *M. blythii* (grey line) in western Eurasia, with the currently accepted subspecific attribution of the different populations; according to Harrison & Lewis (1961), Strelkov (1972), Felten *et al.* (1977) and Palmeirim (1979). (b) An updated geographical distribution of *Myotis myotis* and *Myotis blythii* (this study) in the western part of their distribution range. The broken line indicates the possible eastern extension of the taxon *omari* which is here hypothetically referred to *M. myotis*.

Skull measurements and results of t-tests on interspecific differences. For variable abbreviations, see text. Identification of each individual was assessed by protein analysis

Variable	M. myotis (n = 35)			M. blythii (n = 21)			t	P
	mean	S.D.	range	mean	S.D.	range		
GL	23.26	0.64	21.80–24.10	21.42	0.53	20.34–22.37	11.65	<0.001
ZB	14.69	0.41	13.70–15.56	13.75	0.39	13.08–14.38	8.46	<0.001
BC	9.92	0.23	9.48–10.34	9.63	0.28	9.10–10.18	3.99	<0.001
IC	5.30	0.19	4.95–5.77	5.19	0.13	5.03–5.51	2.54	0.014
CC	6.04	0.29	5.50–6.58	5.67	0.22	5.28–6.01	5.33	<0.001
MM	9.57	0.44	8.69–10.33	8.87	0.28	8.35–9.35	7.23	<0.001
CM^3	9.57	0.36	8.60–10.17	8.67	0.24	8.02–9.01	11.21	<0.001

morphological and ecological adaptations—which are nowadays fixed—and that they do not presently compete with each other.

Ecology: towards a causal speciation model

As understood in the light of present taxonomy, mouse-eared bats show the same species-specific dietary specializations throughout their western distributional range. This probably results from the exploitation of distinct species-specific foraging habitats (Arlettaz, Ruedi & Hausser, 1993). *Myotis blythii* has a larger distributional range than *M. myotis*, occurring from Portugal to Mongolia. As a predator of orthopterans, *M. blythii* appears primarily to be a grassland species (Arlettaz *et al.*, 1993; Arlettaz, 1995). Thus, it is not surprising that its range largely coincides with the warm steppe belt. Moreover, like most elements of the steppe fauna, *M. blythii* has seemingly colonized some secondary grassland habitats (e.g. meadow- and pastureland), although it appears to avoid highly xeric environments and denuded areas. As far as we know, *M. myotis* is restricted to the western Palaearctic region. It shows a much greater latitudinal range than *M. blythii*, occurring from North Africa to the Baltic Sea. Most of its diet consists of ground-dwelling prey. Such prey can be captured in all habitats with open ground, i.e. either park-like mature forests (Rudolph, 1989; Audet, 1990; Arlettaz, 1995), overgrazed pastures or even desert-like habitats (Morocco).

Palaeontological, morphological and genetic studies (Topal & Tusnadi, 1963; Strelkov, 1972; Topal, 1983; Fayard, 1984; Ruedi *et al.*, 1990) suggest that the separation between *M. blythii* and *M. myotis* occurred during the Pleistocene. From the standpoint of cranial morphology, present-day *M. blythii*—the species which has also the widest distribution—seems closer to the ancestral form of mouse-eared bats. Indeed, in eastern Europe, *M. blythii* is already known from Middle Pliocene (Tertiary) deposits (Topal, 1983), whereas it appears only in the late Holocene within south-western European faunas (Sevilla, 1989). In contrast, the first fossil records of *M. myotis* in eastern Europe appear in the Late Pleistocene (Topal & Tusnadi, 1963), and in Spain in the Lower Pleistocene (Sevilla, 1989).

In light of currently available data, the present study offers the first opportunity to propose a coherent—i.e. supported by sound ecological arguments—scenario of speciation for *M. myotis* and *M. blythii*. Sympatric speciation through disruptive selection via habitat specialization and assortative mating (Rice, 1987; Diehl & Bush, 1989) is hardly imaginable for mouse-eared bats owing to their colonial habits; in Europe, under sympatric conditions, most maternity colonies are mixed, as are the

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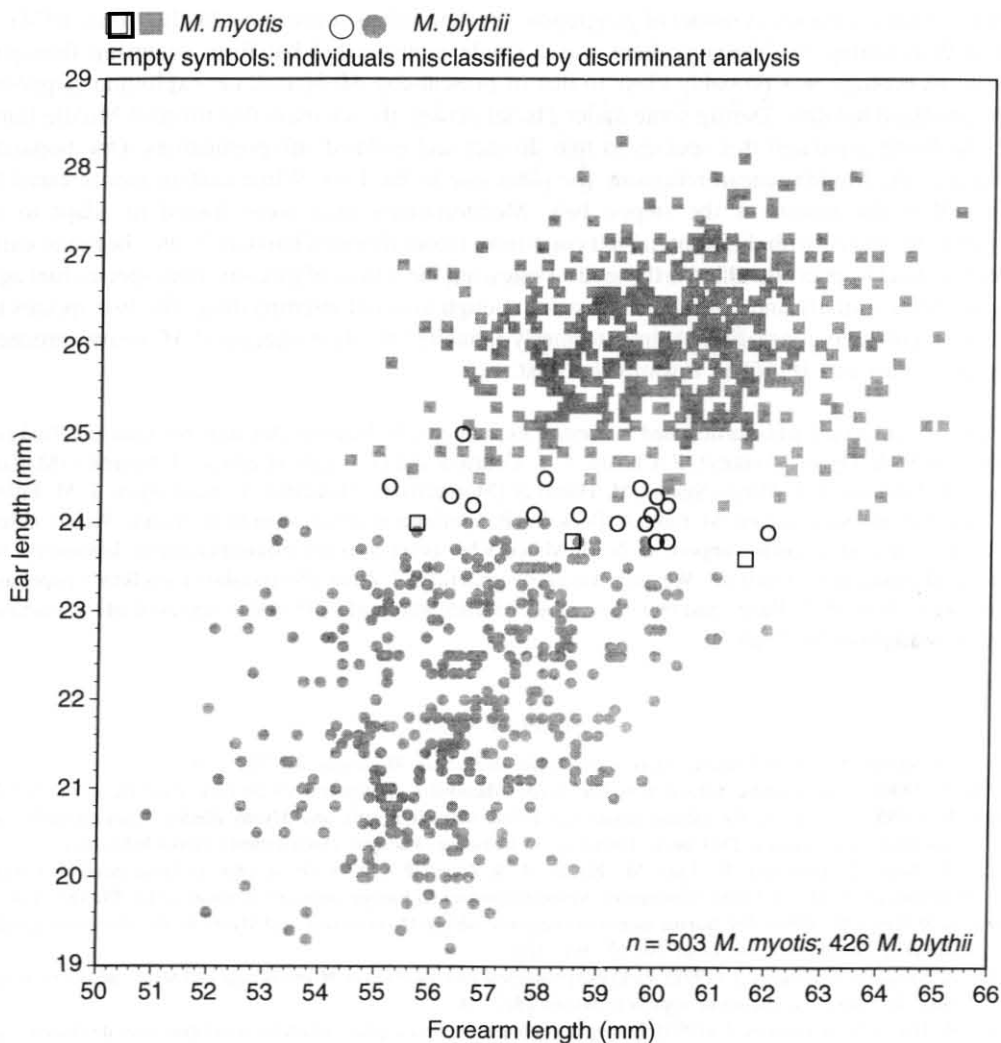Black symbols: *M. myotis*; grey symbols: *M. blythii*
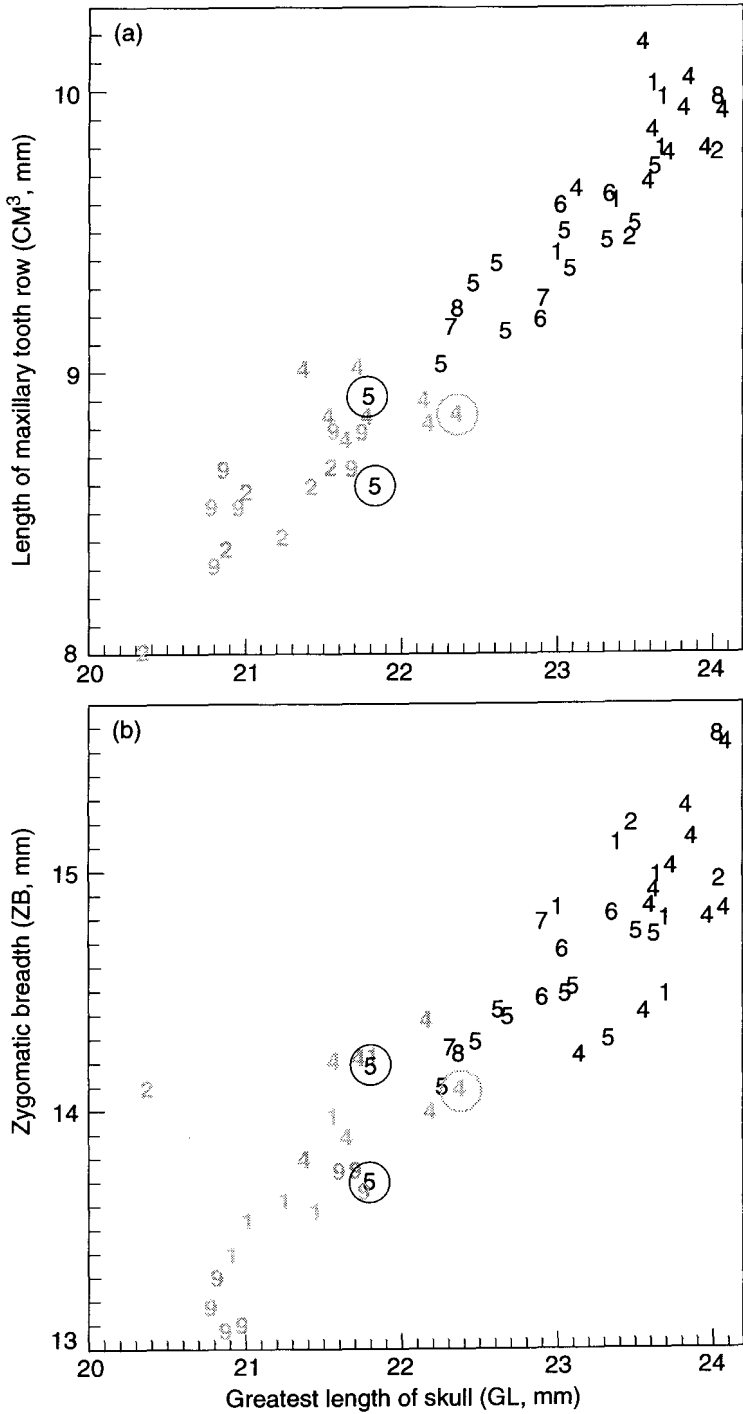
FIG. 7. Relationship between forearm length and ear length for 929 mouse-eared bats measured from various populations. The black symbols indicate the 19 individuals (16 *M. blythii* and 3 *M. myotis*) which were misclassified by the discriminant functions calculated on the whole sample (see text). Misclassified individuals came from Portugal ($n = 8$), SW Switzerland (5), Spain (3), NE Switzerland (2) and Morocco (1).

FIG. 6. Relationship between: (a) greatest skull length (GL) and maxillary tooth row (CM^3); and (b) GL and zygomatic breadth (ZB) for 56 skulls of mouse-eared bats from various origins: 1 = Switzerland; 2 = Cataluña; 4 = Spain (except Cataluña); 5 = Morocco; 6 = Sardinia; 7 = Corsica; 8 = Turkey; 9 = Kirghizstan. The circles indicate the three individuals misclassified by the discriminant function (2 *M. myotis* from Morocco, 1 *M. blythii* from Spain). See text for more details about the discriminant function.

majority of mating roosts. A model of geographic speciation thus seems most likely (Mayr, 1974). We suggest that, during the Tertiary, there was a single mouse-eared bat form occurring throughout Eurasia; its ecology was probably close to that of present-day *M. blythii*, i.e. exploiting steppe-like, warm grassland habitats. During some major glacial period, the ice mass that invaded Middle Europe from the North separated this species in two distinct and isolated sub-populations. One population retreated to the Mediterranean refugium, the other one to the East. While eastern mouse-eared bats could follow the retreat of the steppe belt, Mediterranean ones were forced to adapt to new environments. Faced with different habitat conditions (more denuded habitats?), they began to exploit ground- instead of grass-dwelling arthropods. Following the retreat of glaciers, both species met again, but were behaviourally and ecologically distinct enough to avoid interbreeding. The two species then began to recolonize geographic regions formerly inhabited by their ancestors: *M. myotis* proceeded towards the east and *M. blythii* towards the west.

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