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BIOCHEMICAL ANALYSIS OF RELATIONSHIPS OF MEDITERRANEAN *ALECTORIS* PARTRIDGES

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ABSTRACT.—The Mediterranean *Alectoris* (including *A. rufa, A. graeca, A. chukar,* and *A. barbara*) comprise a group of closely related and morphologically uniform partridges with largely allopatric distributions and instances of natural hybridization in parapatric contact zones. Their taxonomic status and evolution are controversial. We have used multilocus protein electrophoresis to estimate the extent of genetic divergence among nominal *Alectoris* species and within *A. chukar, A. graeca* and *A. rufa*. The average Nei's (1978) genetic distance among conspecific populations ($\bar{D}$ = 0.008; range 0.003–0.021) was 26 times smaller than among species ($\bar{D}$ = 0.208; range 0.071–0.312). The most genetically similar species were *A. rufa* and *A. graeca* ($\bar{D}$ = 0.081); *A. barbara* and *A. chukar* were the most divergent ($\bar{D}$ = 0.303). The F_{st} values among species (F_{st} = 0.75) were more than eight times larger than among conspecific populations (F_{st} = 0.09). The gap in D and F_{st} values for intraspecific and interspecific comparisons indicates a prolonged interruption of gene flow among species and independent evolution of their gene pools. Dendrograms summarizing genetic distance matrices and cladistic analyses of discrete character states suggested that *A. rufa* and *A. graeca* are sister species of recent origin, followed by the most distantly related and ancient *A. chukar* and *A. barbara*. Because protein electrophoresis results are concordant with biogeographical and paleontological information, we construct a hypothesis for the evolution of the Mediterranean *Alectoris*. *Received 2 May 1991, accepted 13 January 1992.*

THE *ALECTORIS* partridges (Galliformes, Phasianidae) are distributed widely in the Palaearctic (Fig. 1). They present intriguing and challenging questions with regard to taxonomic and evolutionary relationships. Peters (1934) included *graeca, chukar,* and *magna* as subspecies of *A. graeca,* a taxonomy that was followed by Dementiev and Gladkov (1952). Voous (1960) largely accepted this classification, but questioned the separate species rank assigned to *A. rufa*. From ethological evidence, Menzdorf (1984) agreed that *graeca, chukar,* and *rufa* had not yet attained true species status. Vaurie (1959), on the contrary, argued that these forms comprised separate species owing to diagnostic differences in facial plumage and vocalizations. This view was supported by Watson (1962a, b), who offered additional evidence of species-specific plumage characters, and of behavioral and ecological separation among parapatric populations. The Vaurie (1959) and Watson (1962a, b) view of seven closely related *Alectoris* species is now widely accepted (Cramp and Simmons 1980, Johnsgard 1988).

Most *Alectoris* species are very similar morphologically, differing only with respect to subtle but diagnostic face and throat plumage patterns (Johnsgard 1988). Their natural ranges (Fig. 1) are largely allopatric, except for sympatry between *melanocephala* and *philbyi* in southern Arabia. Parapatric contact zones exist between *chukar* and *graeca* at the border of Greece and Bulgaria, between *rufa* and *graeca* in the French Alps, and probably between *magna* and *chukar* in central China (Watson 1962a, Bernard-Laurent 1984). Only two zones of overlap and hybridization (sensu Short 1969) have been reported: a well-documented *rufa* and *graeca* hybrid zone in the southern French Alps (Bernard-Laurent 1984); and an unconfirmed *graeca* and *chukar* hybrid zone in Thrace south of the Rhodope Mountains (Dragoev 1974). Extant *Alectoris* populations of Mediterranean and North Atlantic islands probably resulted from human introductions (Watson 1962b, Blondel 1988). The present pattern of only one species per island may represent the outcome of competitive exclusion among two or more species

following repeated introductions (Blondel 1988). Therefore, several lines of evidence, including allopatric distributions, natural hybridization, ecological exclusion, and morphological similarity have been used in support of hypotheses of a recent radiation, and perhaps incomplete speciation, of the Mediterranean *Alectoris* partridges (Voous 1960, Watson 1962a, b, Blondel 1988).

Two conflicting models have been recently proposed to explain evolution and speciation in *Alectoris* (Fig. 1). Watson (1962a) argued that *Alectoris* comprises: (1) the "superspecies" *graeca* (encompassing *graeca,* and *magna*); (2) the "superspecies" *chukar* (encompassing *chukar, philbyi, barbara,* and *rufa*); and (3) *melanocephala,* a separate and more distantly related species. From morphological and biogeographical evidence, Watson argued that *graeca* was the ancestor of the *chukar* lineage, and that *rufa* evolved recently in southwestern Europe after *barbara* crossed the Straits of Gibraltar. Spanò (1975) criticized Watson's model and suggested close relationships of *chukar* with *graeca* and *rufa,* but not with *barbara* and *philbyi.* Bernard-Laurent (1984) and Blondel (1988) cited extant zones of overlap and hybridization for including *graeca, rufa,* and *chukar* in a single superspecies of which *graeca* was the ancestral form. According to these authors, *barbara* never crossed Gibraltar to Europe and is only distantly related to *graeca.*

We used multilocus protein electrophoresis to estimate the extent of genetic divergence among the four Mediterranean species of *Alectoris*: Red-legged Partridge (*A. rufa*), Rock Partridge (*A. graeca*), Chukar (*A. chukar*), and Barbary Partridge (*A. barbara*). In particular, we examined the following hypotheses posed by previous authors: (1) the Mediterranean *Alectoris* speciated very recently (Watson 1962a) and, perhaps, incompletely (Voous 1960, Blondel 1988); (2) *graeca* was the ancestral form of a superspecies from which *chukar* and *rufa* originated at the eastern and western range boundaries, respectively (Bernard-Laurent 1984, Blondel 1988); or, alternatively, (3) *chukar, barbara,* and *rufa* constitute a superspecies, with *rufa* originating from *barbara* after crossing Gibraltar (Watson 1962a). Genetic distances were used to estimate levels of divergence among conspecific populations and among species, and to obtain dendrograms showing phenetic and phylogenetic relationships among species. A tentative calibration of the average rate of protein evolution was used to estimate divergence times, which have been related to current evolutionary and biogeographical hypotheses.

Material and Methods

We analyzed 117 specimens belonging to the following populations: *A. rufa* population 1 ($n = 12$, wild, SW Spain); *A. rufa* 2 ($n = 20$, captive-reared, Italy); *A. graeca* 1 ($n = 10$, wild, E Alps, Italy); *A. graeca* 2 ($n = 20$, captive-reared, Italy); *A. chukar* 1 ($n = 20$, wild, China); *A. chukar* 2 ($n = 5$, captive-reared, Bulgaria); and *A. chukar* 3 ($n = 20$, wild, central Israel); *A. barbara* ($n = 20$, wild, Sardinia, Italy). Captive specimens were obtained from pure-bred, farm-reared stocks. Given the recent foundation of the captive stocks, and their derivation from more than 20 breeding pairs, there was little likelihood that the source populations were subject to substantial inbreeding. Wild birds were collected from localities at which there have been no restocking with reared birds. We also analyzed tissues of Gray Partridge (*Perdix perdix,* $n = 2$) and Ring-necked Pheasant (*Phasianus colchicus,* $n = 2$) as outgroups for rooting phylogenetic trees.

Liver and heart samples were dissected from freshly-killed birds, stored at −20°C for several hours after death, and then stored at −80°C until processing. We separately homogenated about 0.5 g of each tissue in 1 ml of 0.01 M Tris/HCl pH 7.5, 0.001 M Na_2.EDTA, and 0.001 M B-mercaptoethanol buffer and centrifuged for 15 min at 13,000 rpm. Supernatants were diluted in one volume of a 40% glycerol solution, aliquoted in Microtiter plates, and frozen at −80°C until used. Vertical polyacrylamide gel electrophoresis (concentration of 7.5% monomers in the continuous systems) was used to resolve 33 loci. Staining recipes were adapted from Harris and Hopkinson (1976). Electromorphs were presumed to have a simple genetic basis, and were considered as alleles. Alleles were coded by their mobility from the starting line, with the most anodal allele coded as "a."

The BIOSYS-1 program (Swofford and Selander 1989) was used to compute percent polymorphic loci (P) and heterozygosity (H) values. Agreement with Hardy-Weinberg expectations was tested using chi-square analysis (Sokal and Rohlf 1981). Other statistical procedures included: contingency tests of allelic heterogeneity among populations (Workman and Niswander 1970); Nei's (1978) and Rogers' (1972) genetic distance matrices, UPGMA phenograms (Sneath and Sokal 1973); and Wagner networks (Swofford 1981). We have computed F-statistics (Wright 1978) within and among nominal species. These provided two lines of evidence (i.e. genetic distances and F_{st}) on the extent of genetic divergence at different taxonomical levels (Corbin 1987). Cladistic trees were constructed using the program PAUP (Swofford 1985) after coding alleles as present or absent according to the independent-allele model (Buth 1984). The SPSS

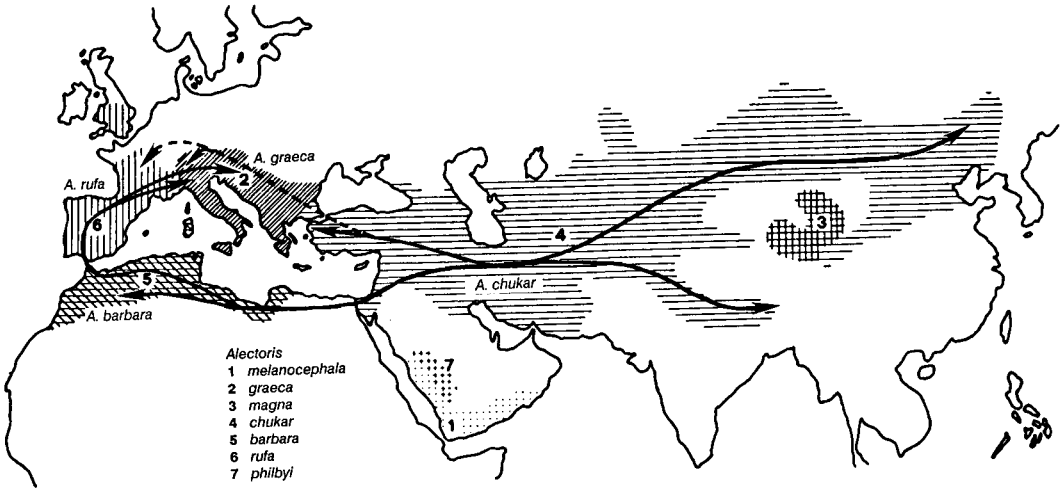

Fig. 1. Distribution of partridges of genus *Alectoris* (adapted from Watson 1962a and Blondel 1988). Continuous lines indicate the evolutionary relationships among species as suggested by Watson (1962a, b). Broken lines indicate evolutionary relationships as hypothesized by Blondel (1988).

(Nie et al. 1975) package was used to compute a Mann-Whitney *U*-test of difference of heterozygosity among populations.

RESULTS

We were able to resolve 33 presumed genetic loci among eight populations of Mediterranean *Alectoris*, and the *Perdix perdix* and *Phasianus colchicus* outgroups (Table 1). Observed heterozygosity ranged from 0.018 (*rufa* 1) to 0.085 (*chukar* 3), and percent polymorphic loci ranged from 6.1 (*barbara*) to 39.4 (*chukar* 2, Table 2). Similar levels of genetic variability have been observed in many other bird species (Corbin 1987, Evans 1987). Heterozygosity did not differ between wild and captive populations of the same species (Mann-Whitney *U*-test, $P < 0.05$). All polymorphic loci were at Hardy-Weinberg equilibrium, excepting sME in *rufa* 2 and PEP-2 in *chukar* 3 ($P < 0.01$; χ^2-test with Levene's [1949] correction for small sample size, and exact probability test). A positive fixation index *F* (Wright 1965) indicated a significant deficiency of heterozygotes in both cases.

Allele frequencies over all polymorphic loci differed significantly among all species ($P < 0.01$; contingency χ^2-test), and among the three populations of *chukar* ($P < 0.01$) and two population of *graeca* ($P < 0.05$). The average F_{st} among species was 0.75, more than eight times higher than the average F_{st} among conspecific populations (0.09).

There were no fixed allelic differences between *rufa* and *graeca*. We found that *chukar* had fixed allelic differences at 12% of loci from *rufa* and *graeca*, while there were 24% fixed allelic differences between *barbara* and the *rufa-graeca* pair. There were 27% fixed differences between *chukar* and *barbara*. Allele frequencies of *rufa* and *graeca* differed significantly (single-locus contingency χ^2-test) at six (18%) of their polymorphic loci. Major differences in allele frequencies between these two species occurred at EST-2, sGOT, and sME. The mean F_{st} between them was 0.572, as compared to only 0.063 and 0.076 among the two *rufa* and the two *graeca* populations, respectively. Intraspecific heterogeneity was greater among *chukar* populations ($\bar{F}_{st} = 0.159$).

Nei's (1978) standard unbiased genetic distances averaged 26 times larger among nominal *Alectoris* species ($\bar{D} = 0.208$; range 0.071–0.312) than among conspecific populations ($\bar{D} = 0.008$; range 0.003–0.021; Table 3). Interspecific genetic distances were lowest between *A. rufa* and *A. graeca*, but even these values ($\bar{D} = 0.081$; range 0.071–0.091) were 18 times larger than the average interpopulation genetic distance within the two species ($\bar{D} = 0.0045$; range 0.003–0.006). The largest genetic distance of the study was between *barbara* and *chukar* ($\bar{D} = 0.303$), which

is one of the highest D-values obtained between what are considered to be congeneric bird species (Zink 1988, Gill and Gerwin 1989).

A phenogram was generated with the unweighted pair-group method using arithmetic averages (UPGMA) of Nei's distances (Fig. 2A). A Wagner network (Fig. 2B) was derived using Rogers' (1972) distances. Nei's D, a nonmetric distance measure, has been widely applied in ornithological research and, therefore, enabled us to compare our results with previous work. Moreover, Nei's D is intended to estimate the proportion of mutational divergence among pairs of lineages, and is related to the time of divergence from the last common ancestor in case of regular rates of molecular evolution (Nei 1978). The two dendrograms were topologically identical. They indicated that *rufa* and *graeca* are most similar, linked at 0.081, followed by *chukar* at 0.168, and *barbara* at 0.282. To construct the Wagner tree, we used *Perdix perdix* and *Phasianus colchicus* as outgroups to root the multiple-addition-criteria network. We optimized branch lengths to maximize goodness-of-fit statistics. Similar lengths among sister branches suggested a regular rate of protein divergence among the lineages. A relative rate test (Beverley and Wilson 1984) was performed using *Perdix perdix* and *Phasianus colchicus* as outgroups. The average ratio of branch lengths among lineages was 1.0133 ± 0.0039, confirming the idea of a regular rate of protein evolution. Parsimony trees (not shown) were derived by cladistic analysis (PAUP) of allele distribution among species. These agreed in topography with the dendrograms shown in Figure 2.

Discussion

Applications of multilocus protein electrophoresis in the study of avian evolution have consistently indicated a comparatively slow rate of genetic divergence among avian taxa. Avise and Aquadro (1982) derived an average Nei's (1972) $\bar{D}$ of 0.08 among 173 congeneric species of birds, an order of magnitude lower than the average of other vertebrates. Variation of pairwise interspecific genetic distances is large, ranging from 0.00 to 0.39 (Gill and Gerwin 1989, Zink and Avise 1990). Many conspecific bird populations are little differentiated, and it is difficult to find local populations or subspecies with F_{st}-values greater than 0.05 and D greater than 0.02 (Barrowclough 1983). Exceptions have been found recently in some Neotropical birds, which had comparatively large genetic distances: (a) among conspecific populations occupying separate Amazon banks (e.g. $\bar{D} = 0.040$ between trans-Amazonian populations of *Pipra coronata*; Capparella 1988); (b) among subspecies (e.g. $\bar{D} = 0.066$ among subspecies of *Chiroxiphia pareola*; Capparella 1988); and (c) among species (Hackett and Rosenberg 1990). These phenomena have been attributed to mechanisms of geographic variation and speciation that may be peculiar to South American birds (Capparella 1988). Hackett and Rosenberg (1990) have suggested the findings may indicate that a reconsideration of the taxonomy of Neotropical birds is warranted.

While theory provides no absolute thresholds of genetic distance for ranking bird taxa (Johnson and Zink 1983), the large body of empirical evidence provides useful guidance. Corbin (1987) described the occurrence of different slopes of the regression line of D on F_{st} between conspecific populations or between species. Large, abrupt differences in D and F_{st} between intraspecific and interspecific levels suggest historical interruption of gene flow among taxa and reorganization of the genomes (e.g. founder effect, random drift, natural selection), and indicate speciation.

The average genetic distance among *Alectoris* ($\bar{D} = 0.208$) was substantially higher than values obtained for most other congeneric bird species (Gill and Gerwin 1989). However, the low average difference among conspecific populations of our study ($\bar{D} = 0.008$) is typical of birds in general, and of galliforms in particular. Interspecific genetic distance between *Lagopus lagopus* and *L. mutus* in Scandinavia was 0.046, as compared to an average of only 0.0035 and 0.0009 among their conspecific populations, respectively (Gyllensten et al. 1985). The average genetic distance among seven populations of *Callipepla californica* was 0.005 (Zink et al. 1987), and their estimated level of gene flow of 5.5 birds per generation was nearly identical to the rate estimated among three populations of *Colinus virginianus* (Ellsworth et al. 1989).

Intraspecific genetic distances among the *Alectoris* populations of this study ranged from 0.003 to 0.021, and were only about 5% of the average interspecific genetic distance of 0.208. The F_{st}-values among *Alectoris* species were more

TABLE 1. Allele frequencies at polymorphic[a] loci in Mediterranean *Alectoris* and outgroups *Perdix perdix* and *Phasianus colchicus*.

Locus (EC no.)	*A. rufa*		*A. graeca*		*A. chukar*			*A. barbara*	*Perdix perdix*	*Phasianus colchicus*	Electrophoretic methods[b]
	1	2	1	2	1	2	3				
ALB	b	b (0.97) c (0.03)	b	b	b	b	b (0.97) c (0.03)	b	a	d	A
H-PT-1	c (0.04) d (0.96)	d	c (0.10) d (0.90)	c (0.05) d (0.95)	b	b (0.70) d (0.30)	b (0.85) d (0.15)	a	c	e	A
H-PT-3	b	b	b	b	b	b	b	b	a	a	A
HB-1	b	b	b	b	b	b	b	b (0.35) c (0.65)	a	a	A
HB-2	b	b	b	b	b	b	b	b	a	a	A
LDH-1 (1.1.1.27)	c	c	c	c	c	c	c	c	a	b	A
LDH-2 (1.1.1.27)	c	c	c	c	c	c	c	c	a	b	A
FUM (4.2.1.2)	a	a	a	a	a	a	a	a	b	b	A
PGM (2.7.5.1)	b	b	b	b	b	b	b (0.93) c (0.07)	b	a	b	B
GDH (1.1.1.47)	b	b	b	b	b	b	b	b	a	a	G
EST-1 (3.1.1.1)	b	b	b	b	c	c	c	d	e	a	C
EST-2 (3.1.1.1)	a	a (0.95) c (0.05)	a (0.40) d (0.60)	a (0.20) d (0.80)	f (0.58) h (0.42)	d (0.10) f (0.70) h (0.20)	d (0.35) e (0.17) f (0.37) g (0.03) h (0.08)	b	i	l	C
EST-3 (3.1.1.1)	b	b	b	b	b	b	b	a	e	d	C
GOT-1 (2.6.1.1)	a (0.08) b (0.92)	a (0.30) b (0.70)	b (0.05) d (0.95)	b (0.35) d (0.65)	e	d (0.10) e (0.90)	d (0.67) e (0.33)	c	f	f	B
GOT-2 (2.6.1.1)	b	b	b	b	b	b	b (0.98) a (0.02)	b	b	b	B
SOD-2 (1.15.1.1)	b	b	b	b	a (0.07) b (0.93)	b	a (0.25) b (0.75)	b	b	b	A
AMY-2 (3.2.1.1)	d	c (0.03) d (0.97)	d	d	d	d	c (0.05) d (0.95)	e (0.35) f (0.65)	a	b	A
ME-1 (1.1.1.40)	b (0.08) c (0.92)	b (0.34) c (0.66)	b (0.95) c (0.05)	b	d	c (0.10) d (0.90)	c (0.05) d (0.95)	a	e	f	B
PEP-2 (3.4.1.1)	a (0.92) c (0.08)	a (0.90) c (0.10)	a	a (0.70) c (0.30)	c (0.70) d (0.30)	c	c (0.77) d (0.23)	a	a	b	G

TABLE 1. Continued.

Locus (EC no.)	*A. rufa*		*A. graeca*		*A. chukar*			*A. barbara*	*Perdix perdix*	*Phasianus colchicus*	Electrophoretic methods[b]
	1	2	1	2	1	2	3				
MPI (5.3.1.8)	b	b	b (0.10) d (0.90)	b (0.10) d (0.90)	b	b	b	c	a	a	A
6-PGD (1.1.1.44)	b	b	a (0.10) b (0.90)	a (0.05) b (0.95)	a (0.30) b (0.70)	b	a (0.22) b (0.78)	b	c	d	F
GOX (1.1.3.1)	b	b	b	b	b	b	b	b	a	b	E
CK (2.7.3.2)	b	b	b	b	b	b	b (0.88) a (0.12)	b	b	b	A
IDH-2 (1.1.1.42)	b	b	b (0.75) c (0.25)	b (0.95) c (0.05)	b (0.98) e (0.02)	b	b (0.95) d (0.05)	b	a	a	B

[a] Monomorphic loci: P-ALB-1,2,3 (A); H-PT-2,4 (A); MDH-1,2 (1.1.1.37; A); PGI (5.3.1.9; B); SOD-1 (1.15.1.1; A).

[b] Electrophoretic system: (A) Disc-Davis pH 8.3 (Davis 1964); (B) Tris-glycine pH 8.5 (Jolley and Allen 1965); (C) Tris-borate pH 8.9 (MacLellan and Ramshaw 1981); (D) Amino-citrate pH 6.1 (Clayton and Tretiak 1972); (E) Tris-citrate pH 8.6 (Clayton and Tretiak 1972); (F) 0.1 M Phosphate pH 7.0 (Harris and Hopkinson 1976); (G) 5,5-Diethylbarbituric acid pH 7.0 (Williams and Reisfeld 1964).

TABLE 2. Genetic variability at 33 loci in Mediterranean *Alectoris* partridges.

Population	Percent polymorphic loci	Heterozygosity	
		Observed	Expected
A. rufa 1	12.1	0.018	0.017
A. rufa 2	18.2	0.029	0.039
A. graeca 1	21.2	0.052	0.051
A. graeca 2	21.2	0.042	0.053
A. chukar 1	15.2	0.050	0.047
A. chukar 2	12.1	0.048	0.042
A. chukar 3	39.4	0.085	0.100
A. barbara	6.1	0.030	0.028

than eight times higher than among conspecific populations. We found *rufa* and *graeca* to be the least divergent species, with an average genetic distance of 0.081, 18 times larger than their average interpopulation distance of 0.0045. Genetic distances and F_{st} within and between species of *Alectoris* are comparable to the values found in Palaearctic and North American birds (Corbin 1987, Evans 1987). The relatively large gap between the population and the species levels suggests an extended interruption of gene flow and an independent evolution among the various *Alectoris* gene pools. The genetic distances ($\bar{D} = 0.303$) between *barbara* and *chukar* populations are very high for congeneric birds. The extent of genetic heterogeneity among *chukar* populations ($F_{st} = 0.159$) is three to five times larger than the values usually observed among conspecific bird populations, and indicates the existence of significant geographic divergence within the *chukar* range.

The pattern of phylogenetic relationships depicted by our dendrograms and cladistic trees (Fig. 2) suggests that *Alectoris* species did not result from contemporaneous episodes of speciation (i.e. as consequence of the fragmentation of an ancestral population). Rather, they arose from at least three waves of speciation. Therefore, the two reported hybrid zones involve sister taxa (*rufa* and *graeca*) and nonsister taxa (*chukar* and *graeca*). The ability to hybridize is most probably attributable to the conservative morpho-anatomical evolution and consequent retention of ancestral characters, rather than to incomplete speciation (McKitrick and Zink 1988, Cracraft 1983, 1989). The rare instances of natural hybridization (which do not compromise the evolutionary independence of the *rufa*, *graeca*, and *chukar* genomes), the large

TABLE 3. Nei's (1978) genetic distances (lower left) and Rogers' (1972) genetic distances (upper right) among Mediterranean *Alectoris* partridges and the outgroups *Perdix perdix* and *Phasianus colchicus*.

		A	B	C	D	E	F	G	H	I	L
A	*A. rufa* 1	—	0.019	0.114	0.108	0.181	0.159	0.183	0.249	0.605	0.572
B	*A. rufa* 2	0.003	—	0.107	0.100	0.177	0.155	0.176	0.245	0.601	0.566
C	*A. graeca* 1	0.091	0.074	—	0.035	0.210	0.194	0.188	0.252	0.592	0.563
D	*A. graeca* 2	0.087	0.071	0.006	—	0.197	0.177	0.179	0.253	0.605	0.563
E	*A. chukar* 1	0.181	0.171	0.211	0.193	—	0.042	0.059	0.284	0.628	0.567
F	*A. chukar* 2	0.154	0.144	0.184	0.162	0.008	—	0.065	0.270	0.626	0.566
G	*A. chukar* 3	0.161	0.151	0.161	0.147	0.021	0.017	—	0.287	0.628	0.571
H	*A. barbara*	0.272	0.263	0.266	0.267	0.312	0.297	0.300	—	0.599	0.568
I	*Perdix perdix*	0.926	0.920	0.898	0.924	0.994	0.990	0.993	0.917	—	0.394
L	*Phasianus colchicus*	0.849	0.838	0.831	0.830	0.839	0.836	0.839	0.843	0.501	—

gap between interpopulation and interspecies genetic distances, and the existence of diagnostic phenotypic characters indicate that the Mediterranean *Alectoris* are composed of what can be considered good evolutionary species.

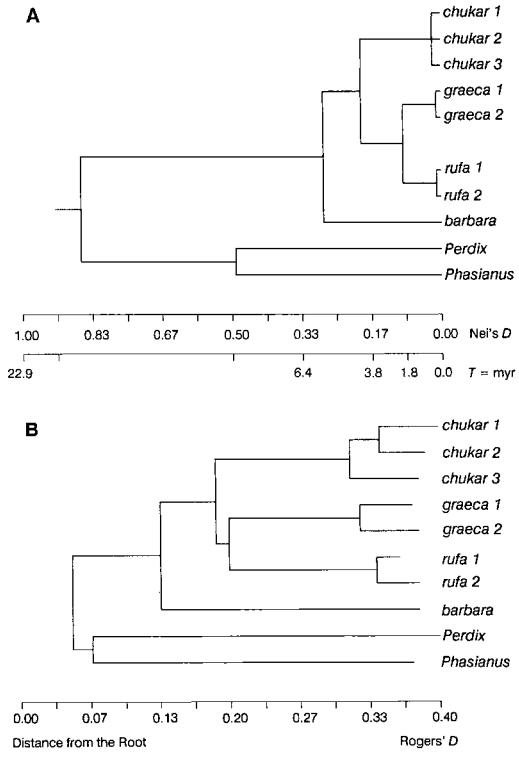

Fig. 2. (A) UPGMA dendrogram of Nei's standard unbiased genetic distances (Table 3) among Mediterranean *Alectoris* and outgroups *Perdix perdix* and *Phasianus colchicus*. Cophenetic correlation is 0.994. Time scale computed using calibration 1 D = 22.9 myr. (B) Wagner tree obtained from Rogers' genetic distances (Table 3). Cophenetic correlation is 0.999.

Comparisons of sister-branch lengths in UPGMA and Wagner dendrograms (Fig. 2), as well as the relative rate test, indicate a relatively constant rate of protein evolution in *Alectoris*. Therefore, we can attempt a calibration of the molecular clock in order to date the divergence times of *Alectoris* species. Based on a galliform fossil (the odontophorin North American quail *Cyrtonyx cooki*), Gutiérrez et al. (1983) proposed Nei's values of 1 D = 23.6 million years (myr). Marten and Johnson (1986) provided a similar estimate of 1 D = 19.7 myr. These rates have proved useful in estimating times of evolutionary branchings in several bird taxa (Johnson and Zink 1983, Zink and Johnson 1984, Randi et al. 1991b), and also approximate the rate of mtDNA evolution in *Ammodramus* (Zink and Avise 1990). From multiple comparisons of nDNA (Helm-Bychowski and Wilson 1986) and enzyme phylogenetic trees, as well as several informative fossils, Randi et al. (1991a) derived the calibration 1 D = 22.9 myr for phasianid birds. Assuming this rate, we derived the following divergence times (Fig. 2A); (1) an initial splitting of the ancestral *Alectoris* into the present *barbara* and *chukar* lineage about 6.4 million years ago (mya); (2) a second splitting of the *chukar* and *graeca-rufa* lineages about 3.8 mya; (3) a final splitting of *graeca* and *rufa* only 1.8 mya.

Our results indicate that genetic divergence among the Mediterranean *Alectoris* is great and that they can be considered good evolutionary species, as proposed by Vaurie (1959) and Watson (1962a, b). Phylogenetic relationships among the species as indicated by our studies do not support the evolutionary scenarios proposed by Watson (1962a) and Blondel (1988): *chukar* is not strictly related to *barbara* and *rufa*; *graeca* is not the ancestral form of a superspecies encom-

passing *rufa* and *chukar*; and *barbara* is not the stem species of *rufa*. Evolution and speciation of *Alectoris* are not recent Pleistocene events as supposed by Watson (1962a); based on our estimated divergence times, speciation events could span from the Miocene-Pliocene boundary to early Pleistocene. The conservative morphology and the small plumage difference are consistent with a slow rate of morphologic evolution and not with recent origins of these species.

We propose the following model of *Alectoris* evolution, substantially concordant with Spanò (1975), and characterized by at least three waves of speciation. About 6 mya, at the Miocene-Pliocene boundary, and ancestral species split into *barbara* and in the *chukar* lineage. This division occurred during a period of climatic warming and aridity that resulted in the closure of the Mediterranean Sea at Gibraltar and its subsequent dessication (Voous 1974). Concurrently, uplift of the Carpathian Mountains created an east-west divide of the central European plains, and separated the Sarmatic Sea from the Mediterranean (Voous 1974). The climate probably favored the spread of birds adapted to arid and steppe habitats, such as *barbara* and *chukar*. We suppose that geologic events resulted in an east-west splitting of the ancestral populations in which the *chukar* lineage spread eastward around the Sarmatic Sea, while *barbara* spread westward along the Mediterranean littoral of the Middle East and Africa. Following speciation, *barbara* eventually crossed the Straits of Gibraltar, and then spread eastward along the European Mediterranean coasts, thereby explaining the presence of fossil *barbara* in France up to the middle Pleistocene (Mourer-Chauvirè 1975). Then, about 4 mya (early Pliocene), the ancestral *chukar* populations, spreading westward, gave rise to the *graeca-rufa* lineage. Finally, at the start of the Pleistocene glaciations, about 1.8 mya, *chukar* populations in western Europe survived in fragmented populations, which probably underwent repeated contraction and expansion according to climatic changes. The fossil record indicates that *barbara* disappeared from Europe during the middle Pleistocene, while both *rufa* and *graeca* occurred in France (Mourer-Chauvirè 1975). The origin of *graeca* and *rufa* was probably fostered by climate-driven contractions of steppe habitat during the early Pleistocene (Blondel 1988). Postglacial warming and extensive deforestation during the Holocene throughout the Mediterranean basin favored expansion of the European and Middle-Eastern populations of *rufa*, *graeca*, and *chukar*. They became parapatric, and originated the actual zones of overlapping and hybridization.

The model is consistent with available fossil, biogeographic and genetic information on the Mediterranean *Alectoris*. It also leads to the following hypothetical predictions for other *Alectoris* species: (1) First, *melanocephala* and *philbyi*, the only two sympatric *Alectoris* (Watson 1962a), arose during the first wave of speciation from anciently separated lineages. They came into extensive contact in the Arabian Peninsula, and evolved character displacement and ecological compatibility (Watson 1962a), allowing their sympatry. From plumage characters (Watson 1962a), we hypothesize that *melanocephala* is related to the *barbara* lineage, and that *philbyi* is related to the *chukar* lineage. (2) Second, *magna* arose from ancient fragmentation of *chukar* populations in China. Plumage similarities between *magna* and *graeca* resulted from convergence rather than from common ancestry. This is in contrast to Watson's (1962a) argument that *magna* is a relic population of a widespread ancestral *graeca* lineage. Our model indicates that *graeca* never reached central Asia. These hypotheses are amenable to testing by expanding biochemical genetic research to all seven extant species of *Alectoris*.

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