The Auk 116(2):324–337, 1999

POPULATION GENETICS OF A HYBRID ZONE BETWEEN THE RED-LEGGED PARTRIDGE AND ROCK PARTRIDGE

ETTORE RANDI[1,3] AND ARIANE BERNARD-LAURENT[2]
[1]*Istituto Nazionale per la Fauna Selvatica, Via Cà Fornacetta 9, I-40064 Ozzano dell'Emilia (BO), Italy; and*
[2]*Office National de la Chasse, CNERA-Faune de Montagne, B.P. 38, F-06201 Nice Cedex 3, France*

ABSTRACT.—We studied the genetic structure of a hybrid zone between Red-legged (*Alectoris rufa*) and Rock (*A. graeca*) partridges in the southern French Alps using six allozyme loci. Allele frequencies showed sharp clinal variation across the hybrid zone, shifting by 60% over a distance of about 60 km, on average. Single-locus clines were coincident, but only partially concordant, with intermediate allele frequencies in the hybrid population. Percent polymorphic loci, heterozygosity, and linkage disequilibria were higher in the hybrids and in some populations near the hybrid zone than in allopatric populations. Nonrandom associations favored parental allele combinations. The hybrids and most of the allopatric populations were in Hardy-Weinberg equilibrium, but six populations near the hybrid zone showed a significant deficit of heterozygotes. The partridges in the hybrid zone included F_1 hybrids, backcrosses, and other recombinant genotypes. This hybrid zone could result from secondary contact of formerly allopatric populations following deglaciation of the Alps, probably not before 6,000 to 8,000 years ago. The width of the observed multilocus cline (70 to 160 km) is much shorter than expected (1,120 to 2,750 km) by neutral diffusion of allelic variants since the time of secondary contact of the two species (2,000 to 3,000 generations). Linkage disequilibria suggest that this hybrid zone is maintained by crossing of genetically divergent parentals and natural selection against hybrid genotypes. Although parapatric interspecific populations can exchange their genes through the hybrid zone, natural selection might constrain gene flow such that two species continue to evolve independently. *Received 12 February 1998, accepted 23 July 1998.*

THE GENETIC STRUCTURE of inter- and intraspecific hybrid zones is usually characterized by abrupt and concordant clinal variation in allele frequencies between the parental populations (Barton and Hewitt 1985, 1989; Harrison 1990, 1993; Hare and Avise 1996). Mosaic hybrid zones (Harrison 1986) can show complex clines owing to differential habitat preferences of the different hybrid genotypes (Rand and Harrison 1989, Howard and Waring 1991, Sites et al. 1995). Hybrid zones appear to be somewhat stable in time and space (Barton and Hewitt 1985, 1989) and could be maintained by dynamic equilibria between gene flow via dispersal and natural selection against hybrid genotypes (Barton and Hewitt 1989). Alternatively, transient hybridization, which results from range expansion and recent contact between reproductively compatible taxa, produces moving fronts of introgression that eventually lead to local genetic extinction of the introgressed populations (Gill 1994, 1997). Hybrids can be intrinsically unfit and then eliminated by selection due to endogenous factors ("tension zones"; Key 1968, Barton and Hewitt 1985, 1989; Searle 1993, Sites et al. 1995). Alternatively or concomitantly, hybrid genotypes can be exposed to exogenous selection pressures in different habitats ("clinal" models; May et al. 1975, Endler 1977) and occasionally can be more fit than parentals at particular transitional locales ("bounded hybrid superiority" model; Moore 1987, Arnold and Hodges 1995, Emms and Arnold 1997).

Hybridization is relatively common in birds (Grant and Grant 1992), and many avian parapatric distributions have been described (e.g. Prigogine 1980, Rising 1983) that apparently represent stable zones of overlap and hybridization (sensu Short 1969). Hybridizing avian populations often show low geographic variation and absence of diagnostic alleles at both nuclear and mitochondrial loci (Barrowclough 1980, Brown and Robbins 1986, Grudzien and Moore 1987, Corbin and Wilkie 1988, Moore et al. 1991, Saino et al. 1992). Morphological traits usually have sharper clinal transitions, which could be controlled by natural and sexual se-

---

[3] E-mail: met0217@iperbole.bologna.it

lection (Moore 1987, Gill 1997). Some avian hybrid zones appear to be stable (Moore and Buchanan 1985), whereas others are transient (Gill 1997) or have shifted their geographic position during historic times (Cook 1975, Rising 1983).

The Red-legged Partridge (*Alectoris rufa*; distributed from Iberia to France and northwestern Italy) and Rock Partridge (*A. graeca*; distributed throughout the Alps, central and southern Apennines and Sicily in Italy, and the Balkans, reaching Albania and Greece; Johnsgard 1988) are largely allopatric but hybridize along the border of the southern French Alps (Bernard-Laurent 1984; Fig. 1). As a consequence of climate deterioration at the Pliocene/Pleistocene boundary, the Red-legged and Rock partridges diverged probably in western and eastern refugia, respectively, and then expanded during the Holocene postglacial warming to a secondary contact zone (Randi et al. 1992, Randi 1996). These two species are genetically well differentiated and probably are sister taxa (Randi 1996). They have diagnostic plumages that allow identification of pure and hybrid birds (Table 1). The morphologically hybrid population presently is distributed over a band of open habitat, about 15 km wide, at moderate elevations along the southern edge of the French Alps (Bernard-Laurent 1984; Fig. 1). In the past, however, the hybrid zone was probably wider, ranging from the northern French Alps to the western Apennines and the Ligurian Alps in Italy (Degland and Gerbe 1867, Spanò 1979).

To date, the genetics of this partridge hybrid zone has not been studied. Here, we report on the genetic structure of the hybrid population relative to Red-legged Partridge and Rock Partridge populations close to and far from the hybrid zone. Results of a survey of six enzyme loci are used to describe single- and multilocus allelic clines and introgression across the hybrid zone, as well as patterns of allele diversity and linkage relationships between loci. Genetic results are integrated with palaeoclimatic, ecological, and behavioral data to suggest the possible origin and dynamics of this hybrid zone.

MATERIALS AND METHODS

During the 1989 to 1993 hunting seasons, we collected 95 Red-legged Partridges and 320 Rock Partridges (hereafter called the "allopatric populations") near or far from their contact zone (Fig. 1). Fourteen allopatric populations were sampled from the southern French and Italian Alps to the eastern Alps. Four other allopatric populations were sampled outside the Alps: two western Red-legged Partridge populations from Portugal and Spain, and two eastern Rock Partridge populations from central Apennines (Italy) and Albania. We collected 38 partridges within the area of species' contact and overlap (the "hybrid zone" across the southern French Alps). The plumage patterns of these birds were analyzed using the criteria of Bernard-Laurent (1984); 30 of the birds were morphological hybrids that exhibited various combinations of species-specific plumage traits (population no. 4; the "hybrid population;" see Fig. 1, Table 2), 7 had typical Rock Partridge plumage (population no. 5; "Rock Partridges within the hybrid zone"), and 1 was a pure Red-legged Partridge (excluded from the following genetic analyses). All birds collected outside the hybrid zone exhibited morphological traits of pure Red-legged or Rock partridges, depending on sampling locations.

Liver and heart tissue samples were collected and stored frozen at −80°C. About 0.5 g of each tissue sample was separately homogenized in 1-mL 0.01 M Tris/HCl pH 7.5, 1 mM Na_2 EDTA, and 1 mM β-mercaptoethanol buffer and centrifuged for 15 min at 17,000 rpm at 4°C. Clear supernatants, diluted in 1 volume of 40% glycerol, were aliquoted in microtiter plates. Polyacrylamide gel electrophoresis was used to study allelic variability of the following six loci, which had high intrapopulation polymorphism and differed in allele frequencies between the two species of partridges (Randi et al. 1992): (1) liver α-naphthylacetate esterase-2 (Est; E.C. No. 3.1.1.1); (2) cytoplasmic glutamate-oxaloacetate transaminase (Got; E.C. No. 2.6.1.1); (3) cytoplasmic malic enzyme (Me; E.C. No. 1.1.1.40); (4) cytoplasmic isocitrate dehydrogenase (Idh; E.C. No. 1.1.1.42); (5) mannose phosphate isomerase (Mpi; E.C. No. 5.3.1.8); and (6) amylase (Amy; E.C. No. 3.2.1.1). Electrophoretic conditions are given in Randi et al. (1992). Electromorphs were treated as alleles and coded by their mobility from the origin, with the most anodal allele named "A," and so forth.

Because intrapopulation allele frequencies did not fluctuate significantly among sampling years (Workman and Niswander's contingency test; data not shown), we pooled all samples collected at the same locality in different years. The electrophoretic data were analyzed using the following software and statistical methods: (1) BIOSYS-1 (Swofford and Selander, 1989) was used to compute allele frequencies, gene-diversity estimates (P = percent polymorphic loci; A = mean number of alleles per locus; H_o and H_e = observed and Hardy-Weinberg expected heterozygosity). We used single-locus chi-square tests of Hardy-Weinberg equilibrium (HWE) with Levene's correction for small sample size and Fisher's

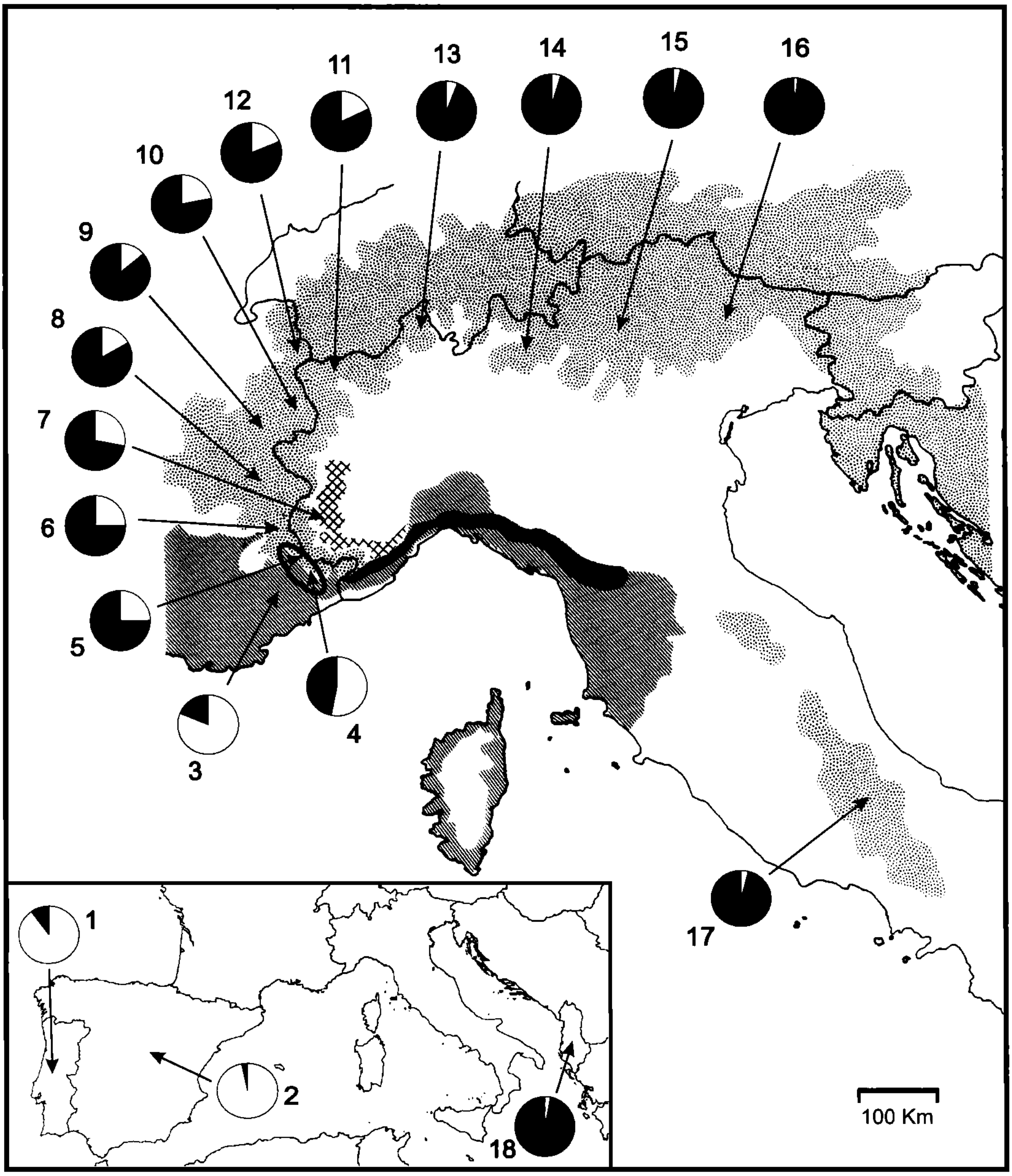

FIG. 1. Distribution of Red-legged Partridges (striped areas) and Rock Partridges (dotted areas) in France and Italy, and distribution of sampled populations. The darker area overlapping part of the Rock Partridge distribution in northwestern Italy indicates the distribution of recently extinct Rock Partridge populations in the western Italian Apennines. The reticulated area indicates the distribution of recently extinct Red-legged Partridge populations in Piedmont (Italy). The present hybrid zone is indicated by an oval. The sampled populations are numbered as follows (numbers 1 to 3 are Red-legged Partridges; numbers 5 to 18 are Rock Partridges): 1 = central Portugal; 2 = central Spain; 3 = Cipieres, France (ca. 20 to 24 km from the hybrid zone); 4 = hybrids from the hybrid zone; 5 = Rock Partridges from the hybrid zone; 6 = the high Valley of Tinée, France (14 to 28 km from the contact zone); 7 = Cuneo, Italy (30 to 40 km from the contact zone); 8 = Champsaur, France (70 to 80 km); 9 = Queyras, France (75 to 85); 10 = Isere, France (80 to 90 km); 11 = Valle d'Aosta, Italy (130 to 150 km); 12 = Savoie, France (170 to 190 km); 13 = Novara, Italy (ca. 250 km); 14 = Sondrio, Italy (ca. 350 km); 15 = Brescia, Italy (ca. 400 km); 16 = Pordenone, Italy (ca. 550 km); 17 = central Apennines, Abruzzo, Italy; 18 = Albania. The pie diagrams indicate the percent of Red-legged Partridge (white) and Rock Partridge alleles (black) found in each population.

TABLE 1. Plumage differences between Rock Partridge and Red-legged Partridge.

Character	Rock Partridge	Red-legged Partridge
Head color	Gray	Forehead gray, rest of head brown
Bar coverts	Black with buff tips	Yellow-buff
Black eye stripe	More than 2 mm wide	Very narrow (<1 mm)
White line over eye	Narrow (1 mm wide)	Line on forehead broadens into eye stripe
Frontal black band	>2 mm wide	Very narrow (<1 mm) or incomplete
White throat patch	Maximum height >5 cm	Smaller (maximum height <5 cm)
Black necklace	Sharply demarcated	Spreads into black streaks on upper chest
Nape	No streak on nape	Some black streaks on nape
Flank feathers	Two black bars	One black bar
Axillary feathers	At least one black bar	No black bar

exact probability test (Weir 1990). Workman and Niswander's (1970) contingency chi-square test was used to assess the significance of allelic heterogeneity among samples. We computed various genetic-distance matrices (D) and used Rogers' D (1972) to obtain UPGMA (Sneath and Sokal 1973) and neighbor-joining dendrograms (NJ; Saitou and Nei 1987). (2) FSTAT and LINKDIS (Black and Krafsur 1985a, b) were used to compute F-statistics (Wright 1965) and to calculate pairwise linkage disequilibrium coefficients within each population estimated as R, the standardized correlation between allele associations. (3) Genetic relationships among populations were represented also through multivariate analyses. NTSYS-pc (Rohlf 1990) was used to obtain the minimum spanning tree (MST) and non-metric multidimensional scaling (MDS) of a principal coordinate analysis (Gower 1966) of the Rogers' D matrix.

RESULTS

Genetic variability of populations.—Allele frequencies (Table 2) diverged significantly between allopatric populations of Red-legged and Rock partridges at Est, Mpi, Got, and Me (Workman and Niswander's contingency test) but not at Amy or Idh. Only one allele, Got-C, was totally absent from one species (Red-legged Partridge) and highly frequent (>0.81) in the other (Rock Partridge) and in hybrid population 4. Some alleles occurred at low frequency in one species and were found in hybrid population 4 and in populations sampled around the hybrid zone. Est-C, Mpi-B, and Idh-B were absent in the Iberian Red-legged Partridge (populations 1 and 2) but frequent in all Rock Partridge populations and present in the hybrid population 4 and in the French Red-legged Partridge (population 3). Got-A, a Red-legged Partridge allele, was present in hybrid population 4, in Rock Partridges within the hybrid zone, and in allopatric populations 6, 9, and 10 near the hybrid zone. Amy-C was present at low frequency in Rock Partridge allopatric populations 6 and 8 and in hybrid population 4. Estimates of gene diversity increased toward the hybrid zone, the mean number of alleles per locus (A) was >2.0, P (0.05 criterion) >50%, $H_o > 0.19$ in all the populations sampled around the hybrid zone (3 to 10; see Fig. 1), and the highest values of H_o and H_e occurred in hybrid population 4. Heterozygosity was higher in French and Italian Rock Partridges sampled near the hybrid zone than in other allopatric Italian and Iberian populations. These findings suggest introgression of alleles through the hybrid zone.

Significant deviations from HWE (Table 3) were observed for some of the loci in six populations near the hybrid zone, and in Red-legged Partridges from Portugal. These birds were collected at four localities (Bombarral, Mertola, Campo Major, Castro Marim) in central and southern Portugal and may represent a heterogeneous sample with different allele frequencies at the different locales (i.e. the Wahlund effect; Hartl and Clark 1990). Nevertheless, they were pooled in a single group because differences in allele frequencies among them have no effects on the description of the genetic clines. Hybrid population 4 was in HWE. All Rock Partridge populations far from the hybrid zone, i.e. east of the Savoie/Valle d'Aosta Alps (populations 13 to 18; see Fig. 1) were in HWE. Significant values of the fixation index F were always due to a deficit of heterozygotes from expected (i.e. positive F values; Table 3).

The genotypic composition of hybrid and allopatric populations was described through the distribution of an individual hybrid index (Barton and Gale 1993):

TABLE 2. Allele frequencies for six polymorphic loci (Est, Mpi, Got, Amy, Idh, Me), gene-diversity estimates, and average values of linkage disequilibrium in 18 partridge populations. A = mean number of alleles per locus; P = percent polymorphic loci (5%); H_o = observed heterozygosity; H_e = Hardy-Weinberg expected heterozygosity; R = coefficient of linkage disequilibrium. See Figure 1 for acronyms and locations of sample populations.

Population	n	Est-A	Est-B	Est-C	Mpi-A	Mpi-B	Got-A	Got-B	Got-C	Amy-A	Amy-B	Amy-C	Idh-A	Idh-B	Me-A	Me-B	A	P	H_o	H_e	R
Red legged-1	20	0.85	0.15	0.00	1.00	0.00	0.00	1.00	0.00	0.30	0.70	0.00	1.00	0.00	0.25	0.75	1.5	50.0	0.100	0.179	0.021
Red legged-2	12	1.00	0.00	0.00	1.00	0.00	0.08	0.92	0.00	0.00	1.00	0.00	1.00	0.00	0.05	0.95	1.3	33.3	0.044	0.043	0.012
Red legged-3	63	0.88	0.02	0.10	0.82	0.18	0.14	0.86	0.00	0.00	1.00	0.00	0.92	0.08	0.31	0.69	2.0	83.3	0.209	0.225	0.091
Hybrid-4	30	0.67	0.00	0.33	0.69	0.31	0.07	0.37	0.57	0.12	0.86	0.02	0.86	0.14	0.60	0.40	2.3	100	0.360	0.401	0.198
Rock-5	7	0.50	0.00	0.50	0.17	0.83	0.07	0.14	0.79	0.00	1.00	0.00	0.93	0.07	0.79	0.21	2.0	83.3	0.190	0.289	0.301
Rock-6	72	0.57	0.01	0.42	0.30	0.70	0.06	0.03	0.92	0.06	0.93	0.01	0.90	0.10	0.88	0.12	2.5	100	0.262	0.269	0.121
Rock-7	8	0.50	0.00	0.50	0.19	0.81	0.00	0.19	0.81	0.06	0.94	0.00	0.69	0.31	0.75	0.25	2.0	100	0.292	0.361	0.307
Rock-8	40	0.32	0.00	0.68	0.30	0.70	0.00	0.04	0.96	0.03	0.94	0.03	0.91	0.09	0.96	0.04	2.2	66.7	0.206	0.214	0.111
Rock-9	53	0.27	0.00	0.73	0.19	0.80	0.01	0.01	0.98	0.04	0.96	0.00	0.84	0.16	0.89	0.11	2.2	66.7	0.196	0.218	0.107
Rock-10	28	0.32	0.04	0.64	0.38	0.62	0.02	0.02	0.96	0.04	0.96	0.00	0.88	0.12	0.84	0.16	2.3	66.7	0.202	0.268	0.289
Rock-11	17	0.62	0.00	0.38	0.09	0.91	0.00	0.00	1.00	0.09	0.91	0.00	1.00	0.00	1.00	0.00	1.5	50.0	0.147	0.136	0.030
Rock-12	8	0.56	0.00	0.44	0.19	0.81	0.00	0.00	1.00	0.06	0.94	0.00	1.00	0.00	1.00	0.00	1.5	50.0	0.146	0.162	0.090
Rock-13	16	0.22	0.00	0.78	0.03	0.97	0.00	0.00	1.00	0.16	0.84	0.00	0.97	0.03	1.00	0.00	1.7	33.3	0.104	0.125	0.070
Rock-14	34	0.06	0.00	0.94	0.16	0.84	0.00	0.00	1.00	0.09	0.91	0.00	1.00	0.00	1.00	0.00	1.5	50.0	0.074	0.092	0.020
Rock-15	6	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.08	0.92	0.00	1.00	0.00	0.92	0.08	1.3	33.3	0.056	0.056	0.020
Rock-16	7	0.00	0.00	1.00	0.17	0.83	0.00	0.00	1.00	0.21	0.79	0.00	1.00	0.00	1.00	0.00	1.3	33.3	0.127	0.111	0.000
Rock-17	24	0.00	0.00	1.00	0.12	0.88	0.00	0.00	1.00	0.02	0.98	0.00	1.00	0.00	0.96	0.04	1.5	16.7	0.049	0.058	0.020
Rock-18	7	0.00	0.00	1.00	0.14	0.86	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.2	16.7	0.048	0.044	0.090

TABLE 3. Loci showing significant ($P < 0.05$) deviations from Hardy-Weinberg equilibrium and probability values obtained using Levene and Fisher chi-square tests. F is the fixation index (Wright 1965).

Locus	Levene test	Fisher test	F
	Red-legged Partridge-1 ($n = 20$)		
Est	0.003	0.030	0.608
Amy	0.000	0.001	0.762
	Red-legged Partridge-3 ($n = 63$)		
Me	0.012	0.017	0.312
	Rock Partridge-5 ($n = 7$)		
Mpi	0.001	0.091	1.000
Got	0.004	ns	0.600
	Rock Partridge-6 ($n = 72$)		
Me	0.021	ns	0.264
	Rock Partridge-7 ($n = 53$)		
Idh	0.005	0.017	0.369
	Rock Partridge-9 ($n = 28$)		
Est	0.020	0.010	0.407
Got	0.000	ns	0.486
	Rock Partridge-1 ($n = 8$)		
Mpi	0.038	ns	0.590

$$z = \sum_i a z_i, \qquad (1)$$

where $a = 1/2n$, n = the number of diagnostic marker genes, and z_i = genotypes generated by the marker genes (indicated as 0, 1, or 2). The individual hybrid index was computed using the frequencies of the most common alleles in the Red-legged Partridge populations (and in the case of loci with three alleles, i.e. the frequency of Est-A and not Est-B+C, and so forth); therefore, values of z can range from 0 (for genetically pure Rock Partridges) to 1 (for pure Red-legged Partridges). Allopatric populations of Rock Partridges (Fig. 2A) and Red-legged Partridges (Fig. 2C) had very skewed distributions of z, with modal values of 0 and 0.9, respectively. The distribution of z within hybrid population 4 (Fig. 2B) was not unimodal and therefore did not indicate a predominance of F_1 hybrids (the central class with 50% genes derived from both the parents). Nor was it strictly bimodal (indicating the complete absence of F_1 individuals), but there were frequent backcrosses (25% and 75% classes) and other recombinants. French Red-legged Partridges and Rock Partridges collected near the hybrid zone (not shown) had a high frequency of intermediate genotypes, which suggests allelic introgression. These findings are consistent with the mixed-phenotype composition of the partridges sampled within the hybrid zone: hybrid, Rock Partridge, and Red-legged Partridge phenotypes accounted for 86%, 10%, and 4%, respectively, of the studied partridges (Bernard-Laurent 1984). Outside the hybrid zone all birds appeared to be phenotypically pure.

Multivariate relationships among populations.—Populations were plotted in a 3-dimensional space (MDS), and a minimum-spanning tree (MST) was superimposed to show relationships among the populations (Fig. 3). Allopat-

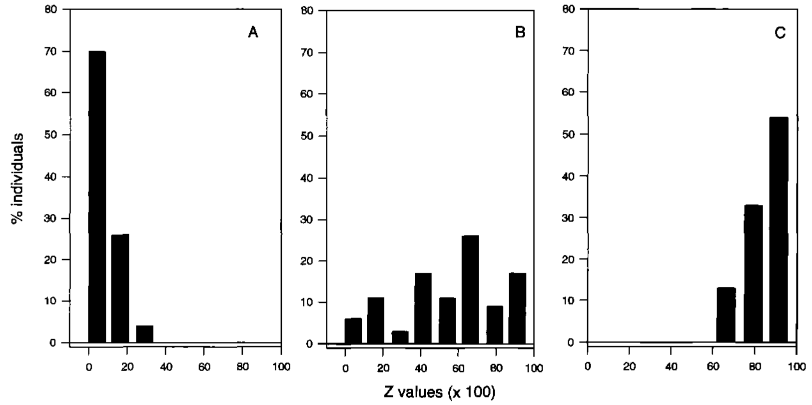

FIG. 2. Distribution of the frequency (%) of the individual hybrid index based on allozyme frequencies ($z \times 100$) in: (A) eastern Rock Partridges (populations 13 to 18); (B) populations 4 and 5 sampled in the hybrid zone; and (C) western Red-legged Partridges (populations 1 and 2). Intermediate values of z indicate F_1 genotypes, whereas extreme values (<25, >75) indicate different backcrosses and recombinant genotypes.

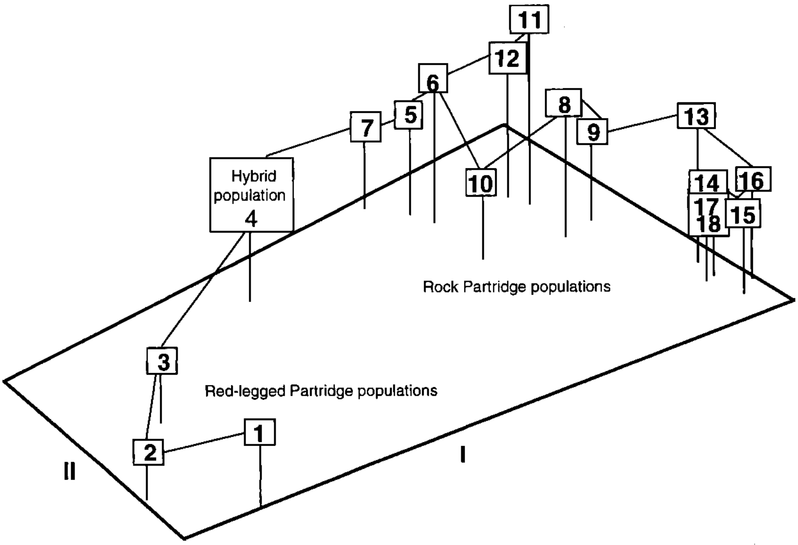

FIG. 3. Three-dimensional distribution of partridge populations obtained by non-metric multidimensional scaling of a principal coordinate analysis (Gower 1966) of the Rogers' D matrix. Populations are connected by a minimum spanning tree. See Figure 1 for acronyms and distributions of populations.

ric Red-legged and Rock partridge populations were completely separated on opposite sides of the first dimension, and the hybrid population was almost intermediate. The tight cluster of Rock Partridges on the right side of the plot encompasses all populations east of Valle d'Aosta and the Savoie Alps (populations 13 to 18) and indicates low levels of genetic divergence among populations. On the contrary, the western Rock Partridge populations (numbers 5 to 12) exhibited increased genetic divergence. MDS also depicted the introgression of Red-legged Partridge alleles into the Rock Partridge populations near the hybrid zone (i.e. populations 5 to 7, which were collected near the hybrid zone, were directly linked to hybrid population 4 through the MST; see Figs. 1 and 3). Dendrograms obtained by clustering Rogers' D matrix with UPGMA and NJ procedures were very similar (not shown) and confirmed the relationships obtained with MDS.

Genetic clines.—Single-locus clines at the four loci that showed significant interspecific divergence (Est, Mpi, Got, Me) were represented by plotting frequencies of the most frequent alleles in the Red-legged Partridge populations against distance of each population from the center of the hybrid zone. Genetic clines showed similar shapes and were roughly coincident, with intermediate and abrupt change of allele frequencies in hybrid population 4. Although frequencies of Got-B and Me-B decayed with exponential regularity far from the hybrid zone, the Est-A and Mpi-A alleles showed greater local departures from the curve. In particular, Rock Partridges from Valle d'Aosta and Savoie (populations 11 and 12) exhibited increased frequencies of Est-A (Fig. 4A). Other populations deviated from a regular clinal pattern, e.g. population 10 had high frequencies of Mpi-A and Got-B (Figs. 4B, 4C), and population 7 had a high frequency of Me-B (Fig. 4D). Pairwise comparisons of allele frequencies in populations 4 to 18 showed that Est and Mpi alleles introgressed into Rock Partridges significantly more than Me and Got alleles, and Mpi significantly more than Me and Got alleles (Fisher's exact test at each locality with sequential Bonferroni adjustment; Rice 1989). These allelic fluctuations suggest differential introgression of Red-legged Partridge alleles into some Rock Partridge populations, although the observed allelic fluctuations could be due to low sample size (i.e. population 7; $n = 8$), or to local isolation and random drift (i.e. population 10).

We computed the hybrid index: HI $= \Sigma(p_i)/4$ (Vanlerberghe et al. 1988) using the most frequent allele (p_i) in Red-legged Partridges at each of the four discriminating loci (Est-A,

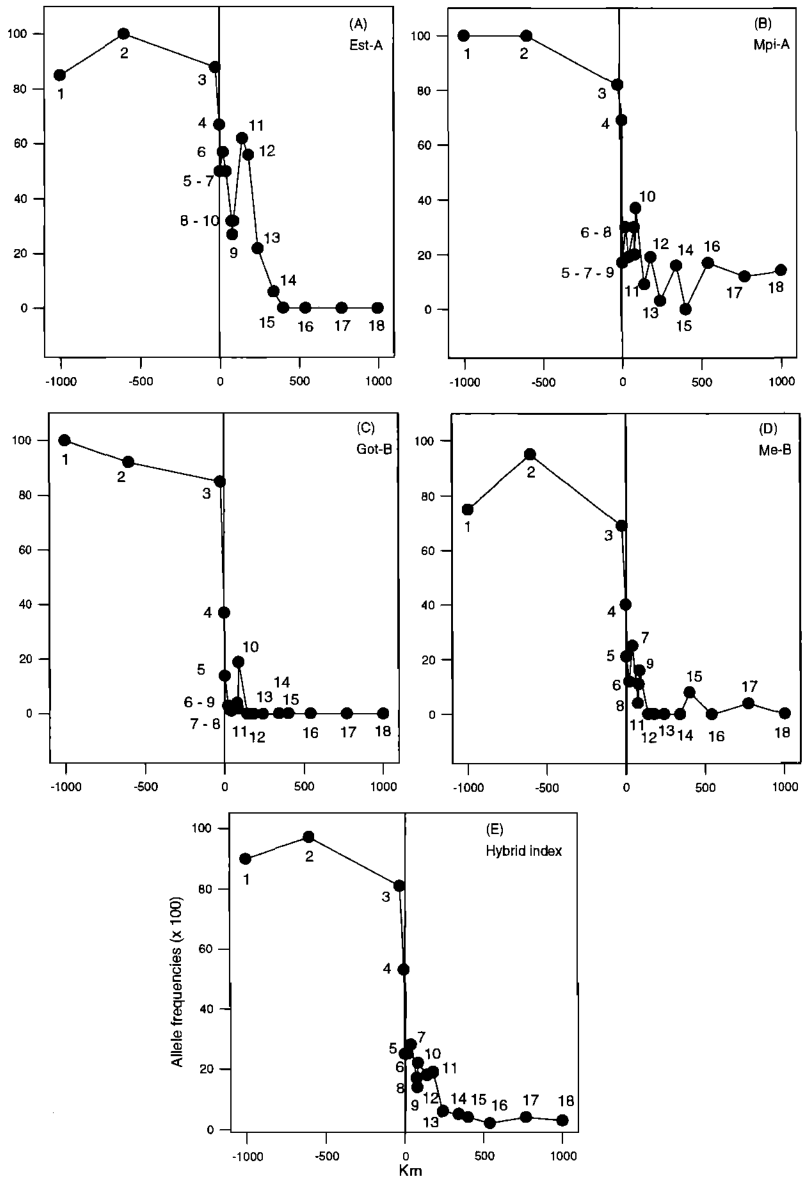

FIG. 4. Clinal variation of four diagnostic alleles in Red-legged Partridge, Rock Partridge, and hybrid populations. Allele frequencies (×100) are plotted against distance (km) of each population from the morphological hybrid zone (0 km). Populations are plotted from east to west, according to the map in Figure 1. The loci are: (A) Est-A, (B) Mpi-A, (C), Got-B, and (D) Me-B; (E) is the plot of the hybrid index computed following Varlenberghe et al. (1988). Distances between populations are approximate measures from the map.

Mpi-A, Got-B, Me-B). Values of HI plotted against distance from hybrid population 4 (Fig. 4E) exhibited a stepped clinal transition from the high frequencies of Red-legged Partridge alleles to the eastern Rock Partridge samples. Hybrid population 4 had HI = 0.53, almost exactly intermediate between the HI of Red-legged Partridges far from the hybrid zone (0.98 in Spain and 0.90 in Portugal) and that of eastern Rock Partridges (0.03 in Albania). The width of the multilocus cline estimated by fitting the observed sigmoid plot to a logistic regression model was $w = 70$ to 80 km, whereas it was $w = 140$ to 160 km when estimated as the distance between HI = 0.78 and 0.22 (corresponding to the difference between 0.80 and 0.20, as corrected following May et al. 1975). Cline width indicates that introgression of Red-legged Partridge alleles into Rock Partridge populations extends far from the geographic location of the hybrid zone, which is only 15 km wide as assessed through the distribution of hybrid phenotypes. These values of HI in fact included some of the populations sampled west of the Savoie / Valle d'Aosta Alps (i.e. populations 6, 7, and 10; Figs. 1 and 4E). Red-legged Partridge and other Rock Partridge populations in France (i.e. 8, 9, 11, and 12) had HI values very close to 0.78 and 0.22, respectively. Therefore, the allozyme cline included some phenotypically pure Rock Partridges as follows: population 6 (collected at ca. 14 to 28 km from the hybrid zone), 7 (collected in Piedmont, Italy, from the eastern slopes of the Southern Alps, ca. 30 to 40 km from the hybrid zone), 10 (collected in Isere, ca. 80 to 90 km from the hybrid zone), and 12 (collected in Savoie, ca. 170 to 190 km from the hybrid zone; see Fig. 1). Introgression of Red-legged Partridge alleles into Rock Partridge populations located within 100 km of the center of the hybrid zone (i.e. populations 6, 7, 8, and 10) involved all polymorphic loci, whereas high HI values of the Savoie (12) and Valle d'Aosta (11) populations were due to the high frequency of Est-A alone (Fig. 4A). The allozyme cline was not defined on the Red-legged Partridge side. It was not possible to sample more French Red-legged Partridge populations because the birds are rare toward the hybrid zone, and sometimes populations are heavily restocked with captive-raised birds.

Linkage disequilibrium.—Analysis of linkage disequilibrium revealed significant *R* coefficients in five of 18 populations (Table 4) owing to nonrandom positive associations among alleles derived from the same parental population. Average pairwise *R* values (Table 2) were plotted against distances from the hybrid zone (Fig. 5A) and compared with the plot of the observed heterozygosities (Fig. 5B). Spacial trends in both estimates corresponded with multilocus and single-locus clines: maximum *R* and H_o values were in or near the hybrid zone. Two Rock Partridge populations (7 and 10) within the area of introgression had *R* values higher than the hybrids (Fig. 5A). We cannot rule out an effect of small sample size on estimated *R* at Cuneo (population 7; *n* = 8), but the Isere sample (population 10; *n* = 28) was larger than the average sample size of this study (*n* = 25).

TABLE 4. Loci with significant ($P < 0.05$) values of pairwise linkage disequilibrium. Alleles of each pair are both indicated in capital letters if conspecific (e.g. row 1, Got-B and Me-B ar highly frequent alleles in Red-legged Partridges), or one in capital and one in lowercase if heterospecific (row 2, Got-B is a Red-legged Partridge allele, and Me-a is a Rock Partridge allele). Observed = the observed frequency (%) of a given allele association; Expected = expected random frequency (%) of a given allele association; *P* = probability of occurrence of deviations between observed and random expected allele associations.

Loci	Alleles	Observed	Expected	*P*
	Red-legged Partridge-3 (*n* = 63)			
Got/Me	B/B	73.5	70.4	0.02
	B/a	29.5	32.6	0.02
	Hybrids (*n* = 30)			
Est/Got	A/B	17.5	14.7	0.04
	A/c	19.0	22.7	0.01
	C/b	4.5	7.3	0.04
	C/C	15.0	11.0	0.01
Got/Me	B/a	8.5	13.2	0.00
	B/B	13.5	8.8	0.00
	C/A	24.0	20.4	0.02
	C/b	10.0	13.6	0.02
	Rock Partridge-5 (*n* = 7)			
Est/Me	A/A	5.5	4.5	0.02
	A/B	2.5	1.5	0.02
	C/A	6.5	5.5	0.02
	C/b	0.5	1.5	0.02
	Rock Partridge-7 (*n* = 53)			
Mpi/Got	A/B	1.0	0.2	0.00
	B/b	0.0	0.8	0.00
	Rock Partridge-9 (*n* = 28)			
EST/Mpi	C/a	10.0	13.5	0.04
	C/B	26.0	22.5	0.04
	B/A	2.0	0.7	0.01
	B/b	0.0	1.2	0.01

DISCUSSION

The Alps represent a barrier to dispersal and an area of secondary contact among many southwestern and southeastern taxa (Taberlet et al. 1998). The slopes of the southern French Alps near the Italian border support several hybrid zones, including those formed by two chromosomal races of a grasshopper (*Podisma pedestris*; Hewitt 1975), two subspecies of crow

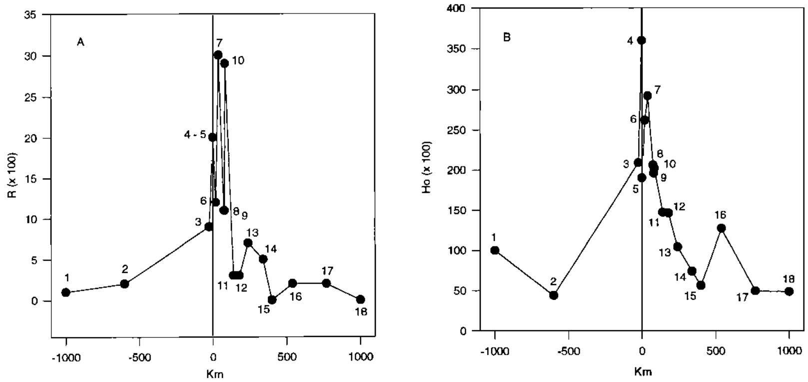

FIG. 5. Plots of the (A) average coefficient of linkage disequilibrium *R* (×100), and (B) observed heterozygosity (×100) across the partridge hybrid zone.

(*Corvus corone*; Saino et al. 1992), and the two species of partridge described in this paper. The Alps were covered by ice during the last glacial maximum, about 18,000 to 20,000 years ago, and most subalpine areas and the central European plains consisted of tundra (Roberts 1989). Alpine and subalpine habitats were not suitable for partridges, which typically inhabit arid flatlands, open hilly slopes, and rocky deforested mountains. Following Holocene warming about 10,000 years ago, ice and tundra retreated, and many plant and animal populations expanded their ranges northward from southern refugia (Hewitt 1989).

Biochemical and molecular analyses (Randi et al. 1992, Randi 1996) suggested that Red-legged and Rock partridges speciated in allopatry about 1.5 to 2.0 millions years ago, at the onset of Pliocene/Pleistocene climatic deterioration. Red-legged and Rock partridges probably were confined to meridional refuge areas in Iberia, Italy, France, and the Balkans, at least until 8,000 to 9,000 years ago, when most of Europe and the Alps were covered by forests. Palaeoecology of the Alps suggests that deforested areas resulting from climatic changes and human activities were widespread from 6,000 to 5,000 years before present (Hewitt 1993). The spread of prehistoric agriculture may have favored the dispersal of partridges that often use pastures, vineyards, orchards, and other cultivated lands (Johnsgard 1988). Thus, the present Red-legged Partridge × Rock Partridge hybrid zone probably originated from a secondary contact in the southern French Alps following the Holocene climatic amelioration, not before 8,000 years ago and probably about 6,000 years ago.

Steep variation in allele frequencies was centered at hybrid population 4, with a shift of values of the multilocus hybrid index HI by 60% over about 60 km distance. Allozyme clines across the partridge hybrid zone were coincident but only roughly concordant, because allele frequencies at some loci fluctuate in populations sampled within about 160 km of the hybrid zone (Fig. 4). The hybrid population had higher heterozygosity and linkage disequilibria than other populations within and near the hybrid zone. Coincident and concordant clines generated by different and non-linked structural loci, as well as linkage disequilibria, are predicted by the tension-zone model of hybrid zones (Barton and Hewitt 1985). The observed allozyme clines in partridges could be maintained through a balance between selection against hybrid genotypes and migration of parental and recombinant individuals. However, the multimodal distribution of the individual hybrid index *z* (Fig. 2), due to the presence of different genotypic classes within the hybrid population, suggests that intrinsic selection against F_1 hybrids within the hybrid zone is weak. Moreover, although the hybrid population was in Hardy-Weinberg equilibrium, other populations near the hybrid zone showed a significant deficit of heterozygotes and linkage disequilibrium, suggesting that natural selection could eliminate some hybrid genotypes and impede gene flow outside the phenotypic hybrid zone.

Variations in the observed shape of the allozyme clines could be due to stochastic fluctuations (e.g. small size of local populations or small sample size) or to selective barriers against gene flow (Barton and Hewitt 1985, Szymura and Barton 1986, Pialek and Barton 1997). The expected width of a cline, as derived from neutral diffusion of allelic variants, is $w = 2.51\sigma T^{0.5}$ (Barton and Gale 1993), where σ is the gene flow computed as the standard deviation of juvenile dispersal, and T is the presumed time in generations since the secondary contact. The observed allozyme cline in partridges has a width of w = 70 to 160 km (Fig. 4E). It is difficult to obtain an estimate of σ, because empirical observations are scarce. From Bernard-Laurent's (1991a) observations on movements of 13 radio-tagged juvenile hybrid partridges, short-term dispersal was σ = 1.0 km, with highly variable individual ranges (0.3 to 25 km). Maximum reported travel distances for Rock Partridges are 25 km in France (Bernard-Laurent, 1991b) and 8 to 9 km in Austria (Hafner 1994). Using values of dispersal of 10 to 20 km per generation, and w = 70 to 160 km, we computed values of T = 5 to 40 generations, corresponding to 10 to 120 years if generation time is 2 to 3 years, that are necessary to produce the observed genetic cline, assuming strict neutrality. Accordingly, the observed multilocus cline should be very recent, and/or the dispersal rate very low, if generated by neutral diffusion of alleles. In other words, neutral diffusion with σ ranging from 10 to 20 km per generation and T of 2 to 3,000 generations since the secondary contact of the two species should have produced a cline of 1,120 to 2,750 km, which is substantially wider than the observed cline of 70 to 160 km. The theory of tension zones offers more rigorous and formal approaches to the study of genetic clines (Szymura and Barton 1986, Mallet et al. 1990, Pialek and Barton 1997), but the incompleteness of our data prevent further elaboration. In particular, we have not sampled more Red-legged Partridges in France because they are scarce around the contact zone, and most localities have been heavily restocked with captive-raised birds. Therefore, because only one side of the hybrid zone was properly sampled, the position, shape, and width of the cline cannot be estimated with greater precision.

In documenting autumn migratory movements of Rock Partridges, Bernard-Laurent (1991b) found that birds were able to fly across the hybrid zone and reach the Red-legged Partridge habitat at lower elevations. Migratory behavior and dispersal suggest a model of formation and dynamics of the partridge hybrid zone: parapatric populations of partridges are separated by boundaries that are located along an area of equilibrium between a cline of decreasing adaptability to environmental conditions (for Red-legged Partridges at higher elevations) and a cline of increasing interspecific competition (for Rock Partridges at lower elevations in the habitat of the other species). This boundary can be crossed, for example, by Rock Partridges that penetrate the Red-legged Partridge areas where they occasionally stay and breed with the resident Red-legged Partridges. The fertility of hybrids has been directly assessed after capturing 13 fertile pairs with hybrid phenotypes (Bernard-Laurent 1990). The multimodal distribution of z suggests that fertile F_1 hybrids can initiate a hybrid population that can backcross and introgress into bordering populations. The stability of the hybrid zone is sustained by the high reproductive success of hybrids (Bernard-Laurent 1987), but the diffusion of hybrids outside the narrow hybrid zone toward the Rock Partridge habitat could be limited by higher winter mortality owing to starvation caused by deep snow (Bernard-Laurent 1988).

Red-legged Partridges and Rock Partridges are widespread, and their distributions are fragmented in patches of allopatric and parapatric populations that are more or less genetically isolated through reduced gene flow among them. The homogenizing effects of introgression through the hybrid zone seem to be strongly counteracted by natural selection against recombinant genomes, which forces the hybrid zone to remain narrow at the present location and acts as a strong barrier to gene flow of Red-legged Partridge alleles into Rock Partridge populations.

ACKNOWLEDGMENTS

We thank P. Leonard, D. Dias, I. Artuso, M. Paganin, and D. Febbo for help in collecting partridges. P. U. Alkon, A. J. Baker, N. Barton, P. Boursot, F. Vanlerberghe, and an anonymous referee provided suggestions that greatly improved an earlier version of the manuscript. Laboratory assistance of A. L. Bolelli and V. Lucchini has been deeply appreciated. This study was supported by the Istituto Nazionale per la Fauna Selvatica Ozzano dell'Emilia (BO), Italy, and Office Nationale de la Chasse, France.

LITERATURE CITED

ARNOLD, M. L., AND S. HODGES. 1995. Are natural hybrids fit or unfit relative to their parents? Trends in Ecology and Evolution 10:67–71.

BARROWCLOUGH, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97:655–668.

BARTON, N. H., AND K. S. GALE. 1993. Genetic analysis of hybrid zones. Pages 13–45 *in* Hybrid zones and the evolutionary process (R. G. Harrison, Ed.). Oxford University Press, New York.

BARTON, N. H, AND G. M. HEWITT. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113–148.

BARTON, N. H, AND G. M. HEWITT. 1989. Adaptation, speciation, and hybrid zones. Nature 341:497–503.

BERNARD-LAURENT, A. 1984. Hybridation naturelle entre Perdrix bartavelle (*Alectoris graeca saxatilis*) et Perdrix rouge (*Alectoris rufa rufa*) dans les Alpes-Maritimes. Gibier Faune Sauvage 2:79–96.

BERNARD-LAURENT, A. 1987. Démographie comparée d'une population de Perdrix bartavelle (*A. g. saxatilis*) et d'une population d'hybrides (*A. g. saxatilis* × *A. rufa rufa*) dans les Alpes-Maritimes. Revue d'Ecologie (Terre Vie) Supplement 4:189–197.

BERNARD-LAURENT, A. 1988. Les déplacement en automne et en hiver de Perdrix rochassière (*Alectoris graeca saxatilis* × *Alectoris rufa rufa*) dans les Alpes méridionales et leurs déterminants. Gibier Faune Sauvage 5:171–186.

BERNARD-LAURENT, A. 1990. Biologie de reproduction d'une population de Perdrix rochassière *Alectoris graeca saxatilis* × *Alectoris rufa rufa* dans les Alpes méridionales. Revue d'Ecologie (Terre Vie) 45:321–344.

BERNARD-LAURENT, A. 1991a. Structure sociale et utilisation de l'espace par la Perdrix rochassière: Variations saisonnières et individuelles. Gibier Faune Sauvage 8:1–30.

BERNARD-LAURENT, A. 1991b. Migrant Rock Partridges in the southern French Alps. Journal für Ornithologie 132:220–223.

BLACK, W. C., AND E. S. KRAFSUR. 1985a. A Fortran program for analysis of genotypic frequencies and description of the breeding structure of populations. Theoretical and Applied Genetics 70:484–490.

BLACK, W. C., AND E. S. KRAFSUR. 1985b. A Fortran program for the calculation and analysis of two-locus linkage disequilibrium coefficients. Theoretical and Applied Genetics 70:491–496.

BROWN, M. J., AND M. B. ROBBINS. 1986. Extensive protein similarity of the hybridizing chickadees *Parus atricapillus* and *P. carolinensis*. Auk 103:667–675.

COOK, A. 1975. Changes in the Carrion/Hooded crow hybrid zone and the possible importance of climate. Bird Study 22:165–168.

CORBIN, K. W., AND P. J. WILKIE. 1988. Genetic similarities between subspecies of the White-crowned Sparrow. Condor 90:637–647.

DEGLAND, D., AND Z. GERBE. 1867. Ornithologie Europeenne. Baillère, Paris.

EMMS, K. S., AND M. L. ARNOLD. 1997. The effect of habitat on parental and hybrid fitness: Transplant experiments with Louisiana irises. Evolution 51:1112–1119.

ENDLER, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, New Jersey.

GILL, F. B. 1994. Rapid, asymmetrical introgression of mitochondrial DNA in hybridizing populations of Blue-winged and Golden-winged warblers. Journal für Ornithologie 135:359.

GILL, F. B. 1997. Local cytonuclear extinction of the Golden-winged Warbler. Evolution 51:519–525

GOWER, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325–338.

GRANT, P. R., AND B. R. GRANT. 1992. Hybridization of bird species. Science 256:193–197.

GRUDZIEN, T. A., AND W. S. MOORE. 1987. Genetic differentiation between the Yellow-shafted and the Red-shafted subspecies of the Northern Flicker. Biochemical Systematics and Ecology 14:451–453.

HAFNER, F. 1994. Das Steinhuhn in Karnten. Okologie, Verhalten und Lebenstraum. Sonderheft 52 der "Carinthia II", Naturwissenachaftlicher Verein für Karnten, Klagenfurt, Austria.

HARE, M. P., AND J. C. AVISE. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). Evolution 50:2305–2315.

HARRISON, R. G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56:337–349.

HARRISON, R. G. 1990. Hybrid zones: Windows on evolutionary process. Oxford Surveys in Evolutionary Biology 7:69–128.

HARRISON, R. G. 1993. Hybrids and hybrid zones: Historical perspective. Pages 3–12 *in* Hybrid zones and the evolutionary process (R. G. Harrison, Ed.). Oxford University Press, New York.

HARTL, D. L., AND A. G. CLARK. 1990. Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts.

HEWITT, G. M. 1975. A sex chromosome hybrid zone in the grasshopper *Podisma pedestris* (Orthoptera: Acrididae). Heredity 35:375–385.

HEWITT, G. M. 1989. The subdivision of species by hybrid zones. Pages 85–110 *in* Speciation and its consequences (D. Otte and J. A. Endler, Eds.). Sinauer Associates, Sunderland, Massachusetts.

HEWITT, G. M. 1993. After the ice: *Parallelus* meets *erythropus* in the Pyrenees. Pages 140–164 *in* Hybrid zones and the evolutionary process (R. G. Harrison, Ed.). Oxford University Press, New York.

HOWARD, D. J., AND G. L. WARING. 1991. Topographic diversity, zone width, and the strength of reproductive isolation in a zone of overlap and hybridization. Evolution 45:1120–1135.

JOHNSGARD, P. A. 1988. The quails, partridges, and francolins of the world. Oxord University Press, New York.

KEY, K. H. L. 1968. The concept of stasipatric speciation. Systematic Zoology 17:14–22.

MALLET, J., N. BARTON, G. M. LAMAS, J. C. SANTISTEBAN, M. M. MUEDAS, AND H. EELEY. 1990. Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. Genetics 124:921–936.

MAY, R. M., J. A. ENDLER, AND R. E. MCMUTRIE. 1975. Gene frequency clines in the presence of selection opposed by gene flow. American Naturalist 109:659–676.

MOORE, W. S. 1987. Random mating in the Northern Flicker hybrid zone: Implications for the evolution of bright and contrasting plumage patterns in birds. Evolution 41:539–546.

MOORE, W. S., AND D. BUCHANAN. 1985. Stability of the Northern Flicker hybrid zone in historical times: Implications for adaptive speciation theory. Evolution 39:135–151.

MOORE, W. S., J. H. GRAHAM, AND J. T. PRICE. 1991. Mitochondrial DNA variation in the Northern Flicker (*Colaptes auratus*, Aves). Molecular Biology and Evolution 8:327–344.

PIALEK, J., AND N. H. BARTON. 1997. The spread of an advantageous allele across a barrier: The effects of random drift and selection against heterozygotes. Genetics 145:493–504.

PRIGOGINE, A. 1980. Etude de quelque contacts secondaires au Zaire oriental. Le Gerfault 70:305–384.

RAND, D. M., AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: Mitochondrial, nuclear and reproductive differentiation of crickets by soil type. Evolution 43:432–439.

RANDI, E. 1996. A mitochondrial cytochrome *b* phylogeny of the *Alectoris* partridges. Molecular Phylogenetics and Evolution 6:214–227.

RANDI, E., A. MERIGGI, R. LORENZINI, G. FUSCO, AND P. U. ALKON. 1992. Biochemical analysis of relationships of Mediterranean *Alectoris* partridges. Auk 109:358–367.

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

RISING, J. D. 1983. The Great Plains hybrid zones. Current Ornithology 1:137–157.

ROBERTS, N. 1989. The Holocene: An environmental history. Basil Blackwell, Oxford.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distances. Studies in Genetics (University of Texas Publications) 7213:145–153.

ROHLF, J. F. 1990. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.7. Exeter Software, Setauket, New York.

SAINO, N., R. LORENZINI, G. FUSCO, AND E. RANDI. 1992. Genetic variability in a hybrid zone between Carrion and Hooded crows (*Corvus corone corone* and *C. c. cornix*, Passeriformes, Aves) in north-western Italy. Biochemical Systematics and Ecology 20:605–613.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

SEARLE, J. B. 1993. Chromosomal hybrid zones in eutherian mammals. Pages 303–353 *in* Hybrid zones and the evolutionary process (R. G. Harrison, Ed.). Oxford University Press, New York.

SHORT, L. H. 1969. Taxonomic aspects of avian hybridization. Auk 86:84–105.

SITES, J. W., JR., N. H. BARTON, AND K. M. REED. 1995. The genetic structure of a hybrid zone between two chromosomal races of the *Sceloporus grammicus* complex (Sauria, Phrynosomatidae) in central Mexico. Evolution 49:9–36.

SNEATH, P. H., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman, San Francisco.

SPANÒ, S. 1979. Nuovi ibridi naturali *Alectoris rufa rufa* (L.) × *Alectoris graeca saxatilis* (Bechstein) sulle Alpi Marittime e relative considerazioni tassonomiche. Annali del Museo Civico di Storia Naturale di Genova 82:154–162.

SWOFFORD, D. L., AND R. K. SELANDER. 1989. BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign.

SZYMURA, J. M., AND N. H. BARTON. 1986. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40: 1141–1159.

TABERLET, P., L. FUMAGALLI, A.-G.WUST-SAUCY, AND J.-F. COSSON. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7:453–464.

VANLERBERGHE, F., P. BOURSOT, J. CATALAN, S. GERASIMOV, F. BONHOMME, B. A. BOTEV, AND L. THALER. 1988. Analyse génétique de la zone d'hybridation entre les deux sous-espèces de souris *Mus musculus domesticus* et *Mus musculus musculus* en Bulgarie. Genome 30:427–437.

WEIR, B. S. 1990. Genetic data analysis. Sinauer Associates, Sunderland, Massachusetts.

WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II: Local genetic differentiation in the Papayo. American Journal of Human Genetics 22:24–29.

WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regards to systems of mating. Evolution 19:395–420.

Associate Editor: A. J. Baker