The Auk 111(2):416–426, 1994

GENETIC STRUCTURE OF CHUKAR (*ALECTORIS CHUKAR*) POPULATIONS IN ISRAEL

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ABSTRACT.—We used multilocus protein electrophoresis to analyze population genetics of Chukars collected at five locations along a north–south gradient in Israel. These birds belonged to two nominal subspecies, the northern *A. c. cypriotes*, and southern *A. c. sinaica*. Chukars from China were used as an outgroup. Allele frequencies at most polymorphic loci differed significantly among Israeli populations, and generally varied according to position along the north–south cline. As compared to northern (more mesic-zone) birds, southern (arid-zone) Chukars had higher gene diversity (*P*-, *H*-, and *A*-values) and significantly less heterozygosity than expected. Clustering and multidimensional scaling of genetic distance matrices revealed a genetic gap between *A. c. sinaica* and *A. c. cypriotes*. Average Nei's unbiased genetic distance was 0.0012 ($\bar{F}_{st}$ = 0.018) within subspecies, and 0.0033 ($\bar{F}_{st}$ = 0.027) between subspecies. The association between geographical distances and pairwise F_{st} among samples was significant (Mantel test). All Israeli birds differed significantly from Chinese conspecifics. Geographically based genetic structuring of Israeli Chukar populations appears surprising, given the country's small size, the countrywide distribution of Chukars, and indirect evidence of moderate to high gene flow among populations (N_m = 6 to 12). However, observed genetic variability corresponds to substantial morphological differences among Israeli Chukars, and reflects the steep climatic gradients and varied landscapes of the country. The observed patterns of variability could have been originated from secondary contact after subspecies isolation in the past. Natural selection and/or metapopulation dynamics may act to maintain genetic structure. Israeli Chukars are a rare avian example of genic divergence across short geographical distances, and comprise a valuable scientific and conservation resource. *Received 29 September 1992, accepted 29 March 1993.*

IN TERMS OF its broad Palearctic distribution and varied habitat affinities, the Chukar (*Alectoris chukar*) may be considered the most successful of the seven *Alectoris* partridges (Cramp and Simmons 1980, Johnsgard 1988). Some 14 *A. chukar* subspecies have been described (Vaurie 1965, Cramp and Simmons 1980, Johnsgard 1988) based primarily on plumage and external morphological traits. The extent and significance of phenotypic divergence among most Chukar subspecies have been poorly investigated. Their geographic ranges are only approximately known, and zones of gradation are rarely described.

In contrast, Chukars of the eastern Mediterranean littoral, and of Israel in particular, have received a fair measure of taxonomic attention. Watson (1962) found distinct clinal morphological variation among eastern Mediterranean Chukars, and recognized three subspecies: the northern, brown-pigmented *A. c. kleini* of Greece; the southern, sand-colored *A. c. sinaica* of the Negev and Sinai deserts; and *A. c. cypriotes* of intermediate plumage coloration and distribution. Vaurie (1965) also recognized two subspecies within the boundaries of Israel: *A. c. cypriotes*, extending from northern Galilee to western Israel, and south to Jerusalem; and *A. c. sinaica*, of the lower Jordan Valley, Negev desert, and Dead Sea depression. Nissani (1974) analyzed a variety of external and skeletal characters among 231 Chukars collected at eight locations in Israel and Sinai. She found considerable geographic variation in morphological traits and strong supporting evidence for the existence of two subspecies in the region, but no unequivocal relationships between morphological and climate variables as predicted by Bergmann's rule.

The taxonomic status of Chukars in Israel appeared unique, and invited genetic analysis. Distinct geographic differences in bird morphology and plumage (Nissani 1974, Alkon unpubl. data) seemed incongruous given the small

size of the country. Moreover, while some geographic variation might be expected in the face of Israel's steep climatic gradients and varied landscapes (Orni and Efrat 1966), the nearly continuous distribution of Chukars in Israel (Paz 1987), and the considerable opportunities for genetic exchange among populations would seem to mitigate against the maintenance of distinct subspecies. The substantial modification of vegetation and landscapes by humans might also be expected to counter environmental selection.

Therefore, we undertook to clarify the genetic status of Chukars in Israel. Our general aim was to assess the extent of genetic divergence among populations by multilocus protein electrophoresis. Specifically, we sought to: (1) evaluate the putative division of Israeli Chukars into two nominal subspecies; (2) determine the genetic basis of geographic phenotypes; and (3) estimate the extent of gene flow among populations. We analyzed tissues of birds collected along a north–south gradient of increasing aridity that included major landscape types and a variety of habitats. We evaluated our findings with respect to natural selection and population dynamics, and with respect to the conservation of genetic diversity.

Materials and Methods

During July 1990, we collected 135 Chukars at locations ranging from the central Negev desert in the south (30°47'N, 34°30'E) to the margin of the Golan Heights in the north (32°48'N, 35°40'E; Fig. 1, Table 1). The mean distance between collection sites was 68 km, and the northernmost and southernmost points were 230 km apart. We sampled five populations from the following geographic regions: (1) border of western and central Negev; (2) central Negev; (3) border of northern Negev and Judean Mountains; (4) border of coastal plain and Shefala (foothills); (5) western margin of Golan Heights. Phytogeographic territories sampled were: (A) Saharo-Arabian (SA) desert; (B) border of SA and Irano-Turanian (IT) steppe; (C) border of IT and Mediterranean (M); and (D,E) Mediterranean (Fig. 1). Vegetation at all sampled locations was at least partly modified by agriculture or other human intervention (Table 1). Birds at sites 1 and 2 were putative *A. c. sinaica*, and those from sites 3, 4 and 5 were putative *A. c. cypriotes* (based on Nissani 1974). Twenty Chukars from an unspecified location in China were used for outgroup comparisons with Israeli birds.

Liver and heart samples were dissected from shot birds (usually in the field), stored in dry-ice coolers, and then deep frozen at −80°C. Tissue samples were transported by air from Israel to E.R.'s laboratory in Italy while stored in dry ice. About 0.5 g of each tissue sample were separately homogenated in 1 ml 0.01 M Tris/HCl, pH 7.5, 0.001 M Na_2.EDTA, 0.001 M β-mercaptoethanol buffer, and centrifuged for 15 min at 13,000 rpm. Supernatants were collected, diluted in 1 volume of 40% glycerol, and aliquoted in Microtiter plates. Polyacrylamide gel electrophoresis was used to resolve 32 putative genetic loci (Table 2). Staining recipes were adapted from Harris and Hopkinson (1976). Electromorphs were presumed to have a simple genetic basis and were treated as alleles. Alleles were coded by their mobility from the starting line, with the most anodal allele coded as "a."

We used the following computer programs to an-

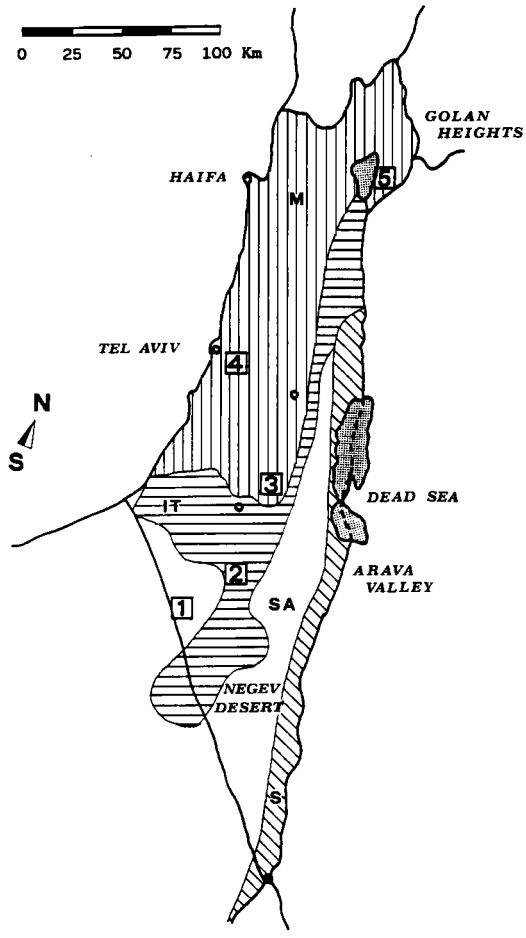

Fig. 1. Map of Chukar sampling localities (1 to 5) in Israel. Phytogeographical regions shown: (M; vertical bars) Mediterranean; (IT; horizontal) Irano-Turanian (steppe); (SA; white) Saharo-Arabian (desert); (S; slanting) Sudanian (subtropical savannah; from Danin 1983).

TABLE 1. Summary description of Chukar collection sites (see Fig. 1).

Population	n	Latitude and longitude	Distance from previous site	Annual rainfall[a]	Habitat
1 Ezuz	17	30°47′N, 34°30′E	—	84 (40%)	Desert semishrub; field crops
2 Sede Boqer	32	30°52′N, 34°47′E	25 km, NE	100 (40%)	Desert semishrub; orchards and field crops
3 Yatir	30	31°20′N, 35°00′E	58 km, NE	278 (33%)	Pine plantation
4 Lod (B-G airport)	27	32°00′N, 34°55′E	73 km, NNW	513 (32%)	Ruderal vegetation (airport)
5 Ramot and Gamla	29	32°48′N, 35°40′E	115 km, NE	464 (25%)	Orchards and field crops; chaparral

[a] In millimeters with CV in parentheses.

alyze genetic data. (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, observed heterozygosity; H_e, Hardy-Weinberg expected heterozygosity; single-locus chi-square tests of Hardy-Weinberg equilibrium with Levene (1949) correction for small sample size, and exact probability test (Weir 1990); Workman and Niswander's (1970) contingency chi-square tests of allelic heterogeneity among samples; F-statistics (Wright 1978); Nei's (1978) and Rogers' (1972) genetic distance (D) matrices; UPGMA phenograms (Sneath and Sokal 1973); and Wagner networks (Swofford 1981). (2) PHYLIP (Felsenstein 1989) was used to compute: least-squares dendrograms, with or without the assumption of a molecular clock (KITSCH and FITCH programs, respectively); and the maximum-likelihood network (MLN) with the CONTML program. (3) NTSYS-pc (Rohlf 1990) was employed to obtain: the minimum-spanning tree (MST; Rohlf 1970) connecting the Rogers' Ds among samples; nonmetrical multidimensional scaling (MDS; Rohlf 1970) of a principal-coordinates analysis (Gower 1966) of the Rogers' D matrix; and a Mantel (1967) test of association between the geographic distances of the sampled populations and their pairwise F_{st} values.

The average rate of gene flow was estimated as N_m, the number of effective migrants among demes per generation, as follows (from Slatkin 1985):

$$N_m = \exp\{[\ln(p1) + 2.44]/(-0.505)\}, \quad (1)$$

where ($p1$) is the mean frequency of "private" (neutral) alleles; or from Takahata and Nei's (1984) modification of Wright (1978),

$$F_{st} = 1/\{1 + 4N_m[n/(n-1)]^2\}, \quad (2)$$

where F_{st} (Wright 1978) is the average among-population proportion of genetic variance.

RESULTS

Allele frequencies were obtained at 16 polymorphic loci of 32 that were resolved (Table 2). A 50% loci polymorphism is one of the highest values reported for birds (Corbin 1987, Evans 1987) and indicates a very high level of genetic diversity among Israeli Chukars. The mean number of alleles per locus (A) ranged from 1.4 to 1.6, and was only 1.2 in Chinese birds (Table 3). Observed heterozygosities (H_o) were lower than expected (H_e) in *sinaica* samples (i.e. 1 and 2). Neither H_o nor H_e differed significantly among samples (t-test; Archie 1985).

Single-locus tests of Hardy-Weinberg equilibrium were conducted using a chi-square test with Levene's (1949) correction for small sample sizes and pooling rare genotypes at loci with multiple alleles. The exact probability test (Weir 1990) also was used. Loci for Israeli populations showing significant departures ($P < 0.05$) from Hardy-Weinberg expectations using the chi-square test (an asterisk indicates case where exact probability test also shows a significant departure) were: (**population 1**) 6PGD*, mIDH*, ADH*, sGOT; (2) PEP-2*, mIDH, ADH; (3) LDH-1*; (4) sGOT; (5) LDH-1*, ACP; (**pooled Israeli populations**) LDH-1*, sME, PEP-2*, mIDH*, ADH*. All the above loci except for sGOT in population 4 had a fixation index (Wright 1978) with a positive sign. The China sample did not show any significant departure from Hardy-Weinberg expectations.

Allele-frequency distributions differed significantly among Israeli populations ($\bar{F}_{st} = 0.04$; Workman and Niswander contingency chi-square = 227.9, df = 96, $P < 0.001$) and were very different between Israeli and Chinese Chukars ($\bar{F}_{st} = 0.17$; $X^2 = 201.8$, df = 25, $P < 0.001$). Allele frequencies at eight loci (6PGD, PGM, sME, PEP-2, mGOT, mIDH, ACP, ADH; 50% of polymorphic loci) were significantly different ($P < 0.01$) among Israeli populations and, with sGOT, were the main contributors to total X^2 and average F_{st} values. Only three loci (sGOT, SOD-2, EST-2) contributed for most (83% of total chi-square value) of the average F_{st} between Israeli and Chinese birds. Estimated divergence

TABLE 2. Chukar allele frequencies for 16 polymorphic loci[a] (with E.C. no. and buffer system[b] in parentheses).

Allele	Population 1	2	3	4	5	China
CK (2.7.3.2; A)						
a	0.059	0.109	0.167	0.093	0.017	0.000
b	0.941	0.891	0.833	0.907	0.983	1.000
ALB (A)						
a	1.000	1.000	0.967	1.000	1.000	1.000
b	0.000	0.000	0.033	0.000	0.000	0.000
LDH-1 (1.1.1.27; A)						
a	0.029	0.000	0.033	0.000	0.034	0.000
b	0.971	1.000	0.967	1.000	0.966	1.000
sGOT (2.6.1.1; B)						
a	0.647	0.750	0.850	0.704	0.879	0.000
b	0.353	0.250	0.150	0.296	0.121	1.000
SOD-2 (1.15.1.1; B)						
a	0.321	0.312	0.383	0.296	0.466	0.075
b	0.676	0.688	0.617	0.704	0.534	0.925
EST-2 (3.1.1.1; C)						
a	0.323	0.312	0.316	0.167	0.190	0.000
b	0.294	0.219	0.183	0.463	0.379	0.000
c	0.265	0.359	0.317	0.296	0.328	0.575
d	0.059	0.047	0.017	0.000	0.017	0.000
e	0.059	0.063	0.167	0.074	0.086	0.425
sMDH (1.1.1.37; A)						
a	1.000	0.984	1.000	1.000	1.000	1.000
b	0.000	0.016	0.000	0.000	0.000	0.000
6PGD (1.1.1.44; D)						
a	0.294	0.359	0.117	0.093	0.103	0.300
b	0.706	0.641	0.883	0.907	0.897	0.700
PGM (2.7.5.1; B)						
a	0.000	0.000	0.000	0.037	0.000	0.000
b	1.000	0.953	0.967	0.852	1.000	1.000
c	0.000	0.047	0.033	0.111	0.000	0.000
sME (1.1.1.40; B)						
a	0.147	0.016	0.000	0.037	0.000	0.000
b	0.853	0.984	1.000	0.963	1.000	1.000
PEP-2 (3.4.1.1; F)						
a	0.118	0.031	0.000	0.000	0.000	0.000
b	0.824	0.813	0.883	0.889	0.966	0.800
c	0.058	0.156	0.117	0.111	0.034	0.200
PEP-3 (3.4.1.1; F)						
a	0.000	0.016	0.000	0.000	0.000	0.000
b	1.000	0.984	1.000	1.000	1.000	1.000
mGOT (2.6.1.1; G)						
a	0.059	0.109	0.017	0.111	0.017	0.000
b	0.941	0.891	0.967	0.889	0.983	1.000
c	0.000	0.000	0.016	0.000	0.000	0.000
mIDH (1.1.1.42; G)						
a	0.853	0.938	0.983	1.000	1.000	0.975
b	0.147	0.062	0.017	0.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	0.025
ACP (3.1.3.2; H)						
a	0.118	0.031	0.000	0.000	0.000	0.000
b	0.118	0.063	0.117	0.019	0.069	0.000
c	0.735	0.828	0.800	0.889	0.931	1.000
d	0.029	0.078	0.083	0.092	0.000	0.000
ADH (1.1.1.1; I)						
a	0.147	0.062	0.033	0.000	0.000	0.025
b	0.853	0.938	0.967	1.000	1.000	0.975

[a] Monomorphic loci (E.C.; buffer system) were: Hb-1(B); Hb-2(B); post-ALB-1 (A); post-Alb-2 (A); H-PT-1 (A); H-PT-2 (A); H-PT-3 (A); LDH-2 (1.1.1.27; A); SOD-1 (1 15.1.1; B); GDH (1.1.1.47; B); EST-1 (3.1.1.1; C); EST-3 (3.1.1.1; C); PGI (5.3.1.9; B); MPI (5.3.1.8; E); sIDH (1.1.1.42; G); FUM (4.2.1.2; A).

[b] References for buffer systems: (A) discontinuous Tris-Glycine pH 8.3 (Davis 1964); (B) discontinuous Tris-Glycine pH 8.5 (Jolley and Allen 1965); (C) Tris-Boric acid pH 8.9 (MacLellan 1982); (D) Phosphate pH 7.0 (Harris and Hopkinson 1976); (E) Tris-Phosphate pH 8.3 (Harris and Hopkinson 1976); (F) Tris-Barbituric acid pH 7.0 (Williams and Reisfeld 1961); (G) Histidine-MES pH 6.1 (MacLellan 1982); (H) HEPES-Imidazole pH 7.4 (MacLellan 1982); (I) Tris-CAPS pH 9.2 (MacLellan 1982).

between Israeli and Chinese Chukars is exceptionally high for conspecific bird populations (Barrowclough 1983, Evans 1987), while average divergence among the Israeli populations is significant but not exceptionally large (Barrowclough and Johnson 1988).

Gene flow patterns, depicted by plotting average allele frequency $p(i)$ against their incidence i/d (Slatkin 1981), suggest a high rate of gene flow among sampled Israeli populations (Fig. 2). Estimates of N_m indicate a generational exchange rate of about 6 (from F_{st}) to 12 (from "private" alleles) birds among the sampled populations.

The pattern of geographical variation of allele frequencies suggests a north–south cline (Fig. 3). Linear regressions of gene diversity estimates (P, A, H_e, H_0) against localities indicates a suggestive but statistically nonsignificant trend towards a reduction of within-sample gene diversity from south to north. These results support the likelihood of geographical structuring of Israeli Chukar populations.

The average Nei's unbiased D was 0.0025 among Israeli populations, and 0.0304 between Israeli and Chinese Chukars. Nei's and Rogers' D matrices (Table 4) were clustered to obtain a summary representation of the relationships among samples. Dendrograms were rooted using the Chinese Chukars as an outgroup. The FITCH dendrogram obtained using Rogers' D

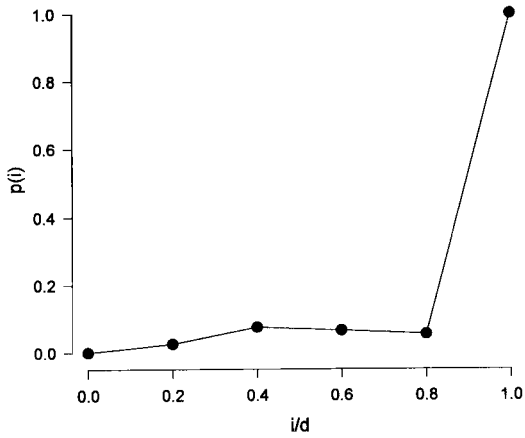

Fig. 2. Pattern of gene flow among five Chukar populations in Israel estimated by Slatkin's (1981) method. Reversed *L*-shaped curve indicates high rate of gene flow.

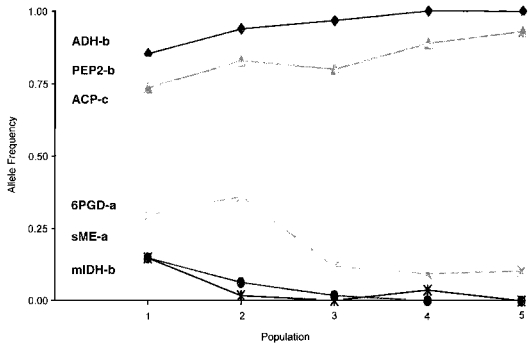

Fig. 3. Geographic variation of allele frequencies at selected loci. Populations as identified in Figure 1.

is shown in Figure 4. Clustering of the Israeli samples revealed a main genetic gap between the *sinaica* (Negev desert) and the *cypriotes* (central and northern Israel) phenotypes. The two *sinaica* samples (1 and 2) were closely related, while among *cypriotes* populations, northern Negev (3) birds were more closely related to Chukars from the Golan Heights (5) than to those from near the Mediterranean coast (4). Similar results were obtained with KITSCH, UPGMA and Wagner dendrograms (not shown) using Nei's and Rogers' *D*s. The same grouping was obtained by clustering the matrix of pairwise F_{st} values between samples (not shown). Significant F_{st} values occurred at the internode linking the cluster of the southern *sinaica* samples with the cluster of central-northern *cypriotes* samples. The average Nei's *D* between subspecies (0.0033) was 64% larger than within subspecies (0.0012); and average between-subspecies F_{st} (0.027) was 35% larger than within-subspecies F_{st} (0.018).

A plot of association between interlocality distances and pairwise F_{st} (Fig. 5) revealed a matrix correlation (*r*; normalized Mantel *Z*) of 0.83. The approximate Mantel *t*-value was 2.51 ($P = 0.01$). From 1,000 random permutations, the one-tailed likelihood to obtain a *Z*-value greater than the observed *Z* was 0.009. Therefore, the overall association between interpopulation distance and F_{st} is highly significant.

To show the eventual effects of high standard errors relative to small genetic distance values on dendrogram topologies, we computed a maximum-likelihood network (MLN) with estimated confidence limits of branch lengths (Fig. 6). Incorporating resultant zero-length branches revealed that: samples 1 and 2 (*sinaica*) are only distantly related to the cluster of 3, 4, and 5; and the latter, in turn, are linked to a hypothetical central "population." To avoid distorting effects of nonlinear relationships on one-

TABLE 3. Genetic-variability estimates at 32 loci in Israeli and Chinese Chukars.[a]

Population	*n*	*A*	*P*	H_o	H_e
			Israel		
1	17	1.6	37.5	0.079 ± 0.026	0.123 ± 0.035
2	32	1.6	43.8	0.094 ± 0.030	0.108 ± 0.032
3	30	1.6	40.6	0.086 ± 0.029	0.091 ± 0.030
4	27	1.4	31.3	0.089 ± 0.030	0.088 ± 0.029
5	29	1.4	28.1	0.059 ± 0.028	0.061 ± 0.028
Pooled	135	1.8	50.0	0.082 ± 0.027	0.094 ± 0.030
			China		
	20	1.2	18.8	0.053 ± 0.026	0.047 ± 0.022

[a] (*n*) = mean sample size per locus; (*A*) = mean number of alleles per locus; (*P*) = percent polymorphic loci; (H_o) = observed mean heterozygosity (±SE); (H_e) = Hardy-Weinberg expected mean heterozygosity (±SE).

TABLE 4. Nei's (1978) unbiased genetic distance (below diagonal) and Rogers' (1972) genetic distance as modified by Wright (1978; above diagonal) for Israeli (1 to 5) and Chinese Chukar populations.

Population	1	2	3	4	5	China
1	—	0.051	0.076	0.077	0.087	0.160
2	0.000	—	0.057	0.067	0.076	0.162
3	0.003	0.002	—	0.062	0.051	0.182
4	0.003	0.002	0.002	—	0.059	0.165
5	0.005	0.004	0.001	0.002	—	0.192
China	0.025	0.027	0.034	0.028	0.038	—

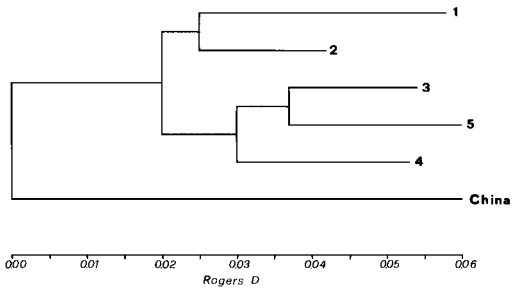

Fig. 4. FITCH dendrogram clustering Rogers' (as modified by Wright 1978) genetic distances for five Chukar populations in Israel and an outgroup of Chinese Chukars (cophenetic correlation = 0.991).

dimensional dendrogram representations, we used Rogers' D matrix to compute an MST and MDS graph in three-dimensional space (Fig. 7). Here again there is: (1) a clear separation of sample groups 1 and 2 from 3–5; and (2) a distinct divergence of sample 4 from the cluster of 3 and 5.

DISCUSSION

Conspecific bird populations studied thus far exhibit only moderate genetic divergence (Barrowclough 1983, Corbin 1987, Evans 1987, Barrowclough and Johnson 1988). Barrowclough (1980) proposed that most bird species (and especially passerines) have demes of large effective size that are connected by substantial gene flow. According to this argument, appearances of distinct local populations and subspecies reflect recent dispersals that would not have experienced sufficient time in isolation to accumulate divergent mutations (Barrowclough 1983). However, although average genetic distance and F_{st} estimates for birds are low, significant levels of genetic heterogeneity (via contingency chi-square tests of enzyme loci) have been reported among conspecific avian populations and nominal subspecies (Gyllensten et al. 1985, Grudzien et al. 1987, Zink et al. 1987, Corbin and Wilkie 1988, Randi et al. 1989). In a few cases, fragmentation and isolation have resulted in F_{st} and D values larger than usual (Yang and Patton 1981, Capparella 1988, Corbin et al. 1988, Hackett and Rosenberg 1990).

By contrast, geographical structuring of conspecific bird populations is rare (but see Randi et al. 1989), and a correspondence of phenotypic with genetic variation has yet to be reported for nominal avian subspecies (Barrowclough and Johnson 1988, Zink 1989). Protein-coding loci may evolve too slowly (Zink and Avise 1990, Randi et al. 1991), at least under selective neutrality (Barrowclough et al. 1985), to provide information on recent phylogeographic histories of bird populations. Studies of the fast-evolving and bottleneck-sensitive mitochondrial-DNA (mtDNA) molecule reveal clearer patterns of genetic variation and geographical structuring (often in accordance with subpecies definitions) than do enzyme data (Shields and Wilson 1987, Avise and Nelson 1989).

Israeli Chukar populations of our study had F_{st}, D, and N_m values similar to average values reported for other birds. In contrast with previous work, they exhibited distinct geographical structuring at polymorphic enzyme loci. About 50% of the polymorphic loci reflected a north-to-south cline of allele-frequency distri-

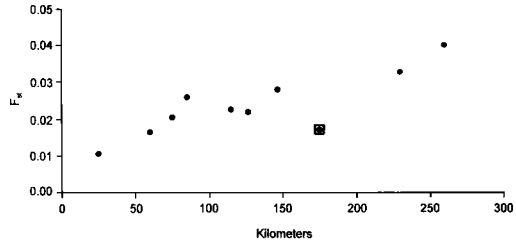

Fig. 5. Association of geographic distances between sample sites with pairwise F_{st} values among five Chukar populations in Israel. Mantel test revealed significant association between matrices. The point that deviates most in plot (squared in figure) represents association between samples 3 and 5, the F_{st} (0.017) of which is lower than expected from the geographical separation of these sites (174 km).

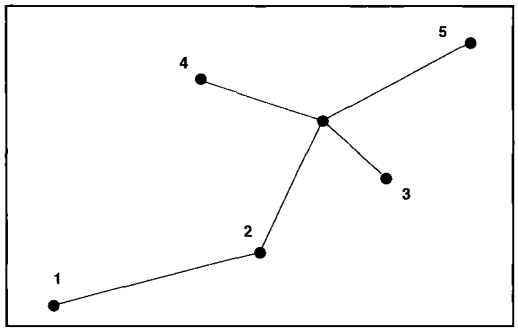

Fig. 6. Maximum-likelihood network (MLN) among five sampled Chukar populations computed by CONTML. Branches with confidence limits including 0 have not been drafted. Network oriented according to approximate geographic location of sampled populations (see Fig. 1).

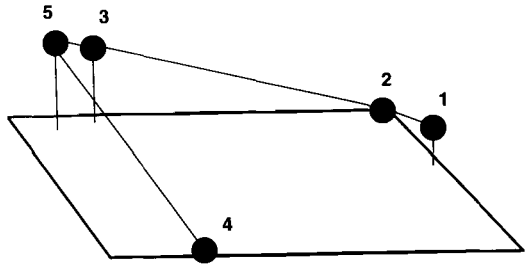

Fig. 7. Nonmetric multidimensional scaling (MDS) with superimposed minimum spanning tree (MST) of Rogers' genetic distances among five Chukar populations in Israel (first two eigenvalues explain 63.7% of total variance; final stress = 0.00092).

butions. These loci contributed substantially to determine the observed values of F_{st} and D. F_{st} and interlocality-distance matrices were significantly correlated. A variety of analyses (i.e. D measures, clustering methods, networks and multidimensional scaling) resulted in concordant representations of genetic relationships among the Israeli samples.

The main genetic gap in F_{st} and D was between samples representing the putative *sinaica* and *cypriotes* subspecies. Our results thereby support the existing nominal subspecific designation of Chukars in Israel. Genetic analysis of Chukars from other areas of Israel would be needed to fully confirm this conclusion, and to delineate boundaries (and possible gradations) of the two subspecies. Major regions not sampled by our study include the upper and lower Galilee, and much of the coastal plain (presumably *A. c. cypriotes* range), and the southern Negev and Arava (Rift) Valley (presumably *A. c. sinaica* range). From morphological and plumage characters of Chukars collected at selected locations, Nissani (1974) placed the border between *sinaica* and *cypriotes* somewhere between Sede Boqer and about 15 km north of Beersheva (Fig. 1). This agrees with our finding of substantial genetic differences between Chukars from Sede Boqer and Yatir (25 km northeast of Beersheva).

Genetic distances and F_{st} values corresponded to (a) geographic distances between populations, and (b) position on a north–south cline that comprises an environmental gradient of increasing aridity. Steep north-to-south and west-to-east climatic gradients exist in Israel. For example, climates in Israel range from "humid to subhumid Mediterranean" to "extremely arid" (Meigs 1954). The mean annual rainfall ranges from more than 1,000 mm at Mt. Meron in the upper Galilee to 30 mm at Eilat in the extreme southern Negev, and from more than 800 mm at Mt. Carmel on the Mediterranean coast in the west to 50 mm at Sedom in the eastern Judean desert (Orni and Efrat 1966, Kadmon 1985). Topographies are diverse—Orni and Efrat (1966) specified 13 major geographical regions in the country—and combine with varied patterns of historical and contemporary land use to produce a mosaic of landscapes that presumably would promote phenotypic and genotypic divergence. However, except perhaps for extremely arid parts of the Negev and Arava (Paz 1987), there appear to be no significant topographical barriers to Chukar dispersion.

With respect to intra-subspecies differences, our findings for *cypriotes* suggest a close relationship between Yatir (3) and western Golan (5) birds, despite their lack of proximity, and a substantial genic separation of near-coastal plain Chukars (4; see Figs. 6 and 7). Both populations 3 and 5 are situated close to the Rift Valley, which may provide an avenue for gene flow. Dispersal and gene flow may be influenced by geographical factors other than gradients, but more complete geographical coverage is required to better delineate genetic relationships among Chukar populations within Israel.

Gene-flow rates estimated from the distribution of "private" alleles and from F_{st} appear moderately high in our populations. Slatkin and Wright models rely on assumptions that are dif-

ficult to meet (i.e. infinite island-population structure, genetic equilibrium, and selective neutrality of allelic variants; Rockwell and Barrowclough 1987). Therefore, N_m values might be overestimated or represent historical, not actual, gene flow. Chukars in Israel do not exibit spatial or altitudinal migrations (Paz 1987) as reported for other locations (Christensen 1970, Johnsgard 1973), and available information for marked Chukars in Israel suggests that movements of individual birds are restricted to a few kilometers in both northern and southern populations (Alkon 1974, Alkon unpubl. data)

The strong geographical structuring of Israeli Chukars suggests restricted effective gene flow or the action of natural selection. Our data are not suited to providing direct support for natural selection. It may be argued that the reduction of observed versus expected Hardy-Weinberg heterozygosity, especially in *sinaica* populations (Table 3), reflects strong natural selection in a stressful desert environment. Indeed, natural selection may affect linked genes controlling fitness characters, and not act directly on enzyme loci. Study populations exhibited a distinct (but not statistically significant) reduction of gene diversity along the north–south cline. Similarly, Nevo and Beiles (1988) found that species from a wide range of taxa (plants, invertebrates and vertebrates) exhibited a general trend towards increased genic diversity from northern to southern Israel. This pattern was attributed to the selective action of stressful environments and to sharp gradients in climate and landscapes. Metapopulation dynamics (i.e. repeated local extinctions and recolonizations) may mimic the effect of gene flow (Slatkin 1985) and produce genic divergences among demes. Recolonization by birds from neighboring populations (such as gene flow in a two-dimensional stepping-stone population model), would generate an isolation-by-distance effect. Metapopulation dynamics may be prominent in severe environments, and our field observations suggest that Negev Chukars undergo large population fluctuations in direct response to climatic conditions (Alkon et al. 1985). Alternatively, the two subspecies could have originated in isolation in refuge areas during the Pleistocene. The actual patterns of distribution and variability could be the consequences of recent dispersal and secondary contact of populations.

Substantial genetic variation in Israeli Chukars parallels a strong geographic variation in phenotypic characters, including body size and plumage pigmentation. Nissani (1974) studied morphological attributes of Chukars collected at seven locations in Israel from the upper Galilee and Golan Heights in the north to the Negev highlands (Sede Boqer) in the south. Body masses and wing lengths of the birds declined from north to south (e.g. mean adult male body mass ranged from 588 g in the upper Galilee to 497 g in the Negev highlands; and mean wing lengths of adults ranged from 167.8 to 160.2 mm at these locations). Multivariate analyses revealed highly significant differences in morphology between Negev highland Chukars and all populations sampled north of Beersheva (Fig. 1). Plumage pigmentation also decreased from north to south, and the light coloration of Negev birds was especially distinctive (Nissani 1974). Other available data generally support Nissani's findings. Mean adult male body mass and wing lengths were 600 $\pm$ SD of 46 g and 172 $\pm$ 4 mm, respectively, for lower Galilee birds (Alkon unpubl. data), and only 515 $\pm$ 44 g and 161 $\pm$ 5 mm, respectively, for Sede Boqer Chukars (Alkon and B. Pinshow unpubl. data). Males Males of both subspecies were about 1.5% larger by mass than females, and sex differences in wing length among adults were statistically significant (Alkon unpubl. data). The grayish-brown dorsal plumage of the Galilee birds contrasted sharply with the light, sandy color of Negev conspecifics. These populations also differ with respect to vocalizations and other behavioral traits (Alkon unpubl. data). Perhaps the most unusual Chukar phenotypes examined by us were Yatir birds (3) of the *cypriotes* complex. They were both large (mean adult male mass = 604 $\pm$ 54 g; D. Meninger unpubl. data) and light-colored, but with a dorsal pigmentation that appeared lustrous as compared to the flat, sandy shade of Negev birds.

In summary, available information indicates that larger overall body size (i.e. body mass and wing length) of *cypriotes* versus *sinaica* birds is a principal phenotypic character that corresponds to genetic differences among Israeli Chukars. The unusual pigmentation of Yatir birds suggests that plumage color may not consistently correspond to genetic subspecific distinctions. Nissani's (1974) findings indicate that sizes of other morphological structures (e.g. bill and leg measurements) decline along a north–south cline for Israeli birds, but that putative

sinaica specimens from the mountains of southern Sinai were substantially larger than their Negev conspecifics.

The observed phenotypic and genetic variations among Israeli Chukars apparently are maintained despite substantial human modification of landscapes and habitats. Many recent human changes, moreover, should act to ameliorate severe environmental conditions, and thereby minimize differences in habitat suitability across landscape and geographic clines. For example, three collection sites of our study, including both *sinaica* locales, contained: areas of irrigated agriculture (1, 2, and 3); one site was a planted pine forest in a semi-arid landscape (3); and the last comprised ruderal vegetation of a highly disturbed area (4; Table 1). The maintenance of strong genetic and phenotypic distinctions in the face of such landscape modifications does not argue for sensitive and rapid responses to environmental selective forces among Israeli Chukars. Conversely, the extent of environmental modification (or the time elapsed) may not have sufficed to mitigate the consequences of historical biogeographical factors or the action of strong natural selection and the metapopulation phenomena that promote genetic diversity.

The strong genetic structuring of Israeli Chukar populations across small geographic distances is unique among birds. Israeli Chukars, therefore, comprise a valuable resource both scientifically (for genetic and evolutionary research) and with respect to the conservation of *Alectoris* genomes. Moreover, the large genetic distance between Israeli and the Chinese Chukars indicates an extensive divergence in world Chukar populations. We recommend that management of Chukar in Israel recognize the importance of local populations and should be aimed at maintaining population integrity and welfare. As a first step in defining an appropriate management program, we recommend that: (a) the abundance and genetic status of populations be regularly monitored; (b) hunting regulations reflect the existence of specific populations; and (c) Chukar stocking and rearing programs avoid the risk of mixing extant population genomes.

ACKNOWLEDGMENTS

We thank the Israel Nature Reserves Authority (NRA) for facilitating this research, and the following NRA staff for help in collecting field samples or other assistance: Doron Akiva, Brian Evans, Eitan Feldman, Eliazar Frankenburg, Ysrael Hershkovitz, David Meninger, Uriel Safriel, David Shevah, and Yacov Shmuel. David Meninger also provided unpublished Chukar morphologic data. Other individuals who assisted in sample collection and processing were Arieh Ohad, Sayid Daher, Yosef Steinberger, and especially Stephan G. Kohlmann. Weather data were provided by Sara Rubin of the Israel Metereological Service. Colleagues at the Jacob Blaustein Institute for Desert Research provided help, including Barry Pinshow (Chukar morphologic data), Nurit Winkler (translation of Hebrew documents), and staff members of the Desert Meteorology Unit (climate data). Support for E.R.'s stay in Israel was provided by the Blaustein International Center for Desert Studies and the Istituto Nazionale per la Fauna Selvatica, Bologna, Italy. This is publication no. 172 of the Mitrani Center for Desert Ecology.

LITERATURE CITED

ALKON, P. U. 1974. Social behavior of a population of Chukar Partridge (*Alectoris chukar cypriotes*) in Israel. Ph.D. thesis, Cornell Univ., Ithaca, New York.

ALKON, P. U., A. A. DEGEN, B. PINSHOW, AND P. J. SHAW. 1985. Phenology, diet, and water turnover rates of Negev desert Chukars. J. Arid Environ. 9:51–61.

ARCHIE, J. W. 1985. Statistical analysis of heterozygosity data: Independent sample comparisons. Evolution 39:623–637.

AVISE, J. C., AND W. S. NELSON. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. Science 243:664–648.

BARROWCLOUGH, G. F. 1980. Gene flow, effective population size, and genetic variance components in birds. Evolution 34:789–798.

BARROWCLOUGH, G. F. 1983. Biochemical studies of microevolutionary processes. Pages 223–261 *in* Perspectives in ornithology (A. H. Brush and G. A. Clark, Jr., Eds.). Cambridge Univ. Press, Cambridge.

BARROWCLOUGH, G. F., N. K. JOHNSON, AND R. M. ZINK. 1985. On the nature of genic variation in birds. Curr. Ornithol. 2:135–154.

BARROWCLOUGH, G. F., AND N. K. JOHNSON. 1988. Genetic structure of North American birds. Pages 1630–1638 *in* Acta XIX Congressus Internationalis Ornithologici (H. Ouellett, Ed.). Ottawa, Ontario, 1986. National Museum Natural Science.

CAPPARELLA, A. P. 1988. Implications for the speciation process. Pages 1658–1664 *in* Acta XIX Congressus Internationalis Ornithologici (H. Ouellett, Ed.). Ottawa, Ontario, 1986. National Museum Natural Science.

CHRISTENSEN, G. C. 1970. The Chukar Partridge: Its introduction, life history, and management. Nevada Dep. Fish and Game, Biol. Bull. 4.

CORBIN, K. W. 1987. Geographic variation and speciation. Pages 321–353 *in* Avian genetics. A population and ecological approach (F. Cooke and P. A. Buckley, Eds.). Academic Press, London.

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

CORBIN, K. W., B. C. LIVEZEY, AND P. S. HUMPHREY. 1988. Genetic differentiation among steamer-ducks (Anatidae: *Tachyeres*): An electrophoretic analysis. Condor 90:773–781.

CRAMP, S., AND K. E. L. SIMMONS (Eds.). 1980. Handbook of the birds of Europe, the Middle East and North Africa. Vol. 2, The birds of the western Palearctic. Oxford Univ. Press, Oxford.

DANIN, A. 1983. Desert vegetation in Israel and Sinai. Cana Publishing House, Jerusalem.

DAVIS, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404–427.

EVANS, P. J. H. 1987. Electrophoretic variability of genes products. Pages 105–162 *in* Avian genetics. A population and ecological approach (F. Cooke and P. A. Buckley, Eds.). Academic Press, London.

FELSENSTEIN, J. 1989. PHYLIP 3.2 manual. Univ. California Herbarium, Berkeley.

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

GRUDZIEN, T. A., W. S. MOORE, J. R. COOK, AND D. TAGLE. 1987. Genic population structure and gene flow in the Northern Flicker (*Colaptes auratus*) hybrid zone. Auk 104:654–664.

GYLLENSTEN, U., N. RYMAN, AND T. SAETHER. 1985. Genetic divergence between Willow Grouse (*Lagopus lagopus* L.) and Rock Ptarmigan (*Lagopus mutus* L.) and the genetic structure of Scandinavian grouse populations. Hereditas 102:47–55.

HACKETT, S. J., AND K. V. ROSENBERG. 1990. Comparison of phenotypic and genetic differentiation in South American antwrens (Formicariidae). Auk 107:473–489.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co., Amsterdam.

JOHNSGARD, P. A. 1973. Grouse and quails of North America. Univ. Nebraska Press, Lincoln.

JOHNSGARD, P. A. 1988. The quails, partridges, and francolins of the world. Oxford Univ. Press, Oxford.

JOLLEY, W. B., AND H. W. ALLEN. 1965. Formation of complexes between basic proteins of leucocytes and plasma globulins. Nature 208:390–391.

KADMON, N. (Chief Ed.). 1985. Atlas of Israel, 3rd ed. Survey of Israel and Carta, Tel-Aviv.

LEVENE, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91–94.

MACLELLAN, T. 1982. Electrophoretic buffers for PAGE gels at various pH. Anal. Biochem. 126:94–99.

MANTEL, N. A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

MEIGS, P. 1954. Design and use of homoclimatic maps: Dry climates of Israel as an example. Pages 99–111 *in* Desert research. Research Council of Israel, Spec. Publ. 2, Jerusalem.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

NEVO, E., AND A. BEILES. 1988. Genetic parallelism of protein polymorphism in nature: Ecological test of the neutral theory of molecular evolution. Biol. J. Linn. Soc. 35:229–245.

NISSANI, R. 1974. Geographic variability of the Chukar *Alectoris chukar* (Gray) and Bergmann's rule. M.Sc. thesis, Hebrew Univ., Jerusalem.

ORNI, E., AND E. EFRAT. 1966. Geography of Israel. Israel Program for Scientific Translation, Jerusalem.

PAZ, U. 1987. The birds of Israel. Steimatzky Ltd., Tel-Aviv.

RANDI, E., F. SPINA, AND B. MASSA. 1989. Genetic variability in Cory's Shearwater (*Calonectris diomedea*). Auk 106:411–417.

RANDI, E., G. FUSCO, R. LORENZINI, AND T. M. CROWE. 1991. Phylogenetic relationships and rates of allozyme evolution within the Phasianidae. Biochem. Syst. Ecol. 19:213–221.

ROCKWELL, R. F., and G. F. BARROWCLOUGH. 1987. Gene flow and the genetic structure of populations. Pages 223–255 *in* Avian genetics. A population and ecological approach (F. Cooke and P. A. Buckley, Eds.). Academic Press, London.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, Univ. Texas Publ. 7213:145–153.

ROHLF, F. J. 1970. Adaptive hierarchical clustering schemes. Syst. Zool. 19:58–82.

ROHLF, F. J. 1990. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.60. Exeter Software, Setauket, New York.

SHIELDS, G. F., AND A. C. WILSON. 1987. Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNA's. Evolution 41:662–666.

SLATKIN, M. 1981. Estimating levels of gene flow in natural populations. Genetics 95:323–335.

SLATKIN, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393–430.

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

SWOFFORD, D. L. 1981. On the utility of the distance Wagner procedure. Pages 25–43 *in* Advances in cladistics: Proceedings of the first meeting of the

Willi Hennig Society (V. A. Funk and D. R. Brooks, Eds.). New York Botanical Garden, Bronx, New York.

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.

TAKAHATA, N., AND M. NEI. 1984. F_{st} and G_{st} statistics in the finite island model. Genetics 107:501-504.

VAURIE, C. 1965. The birds of the Palearctic fauna. Non-Passeriformes. H. F. and G. Witherby, London.

WATSON, G. E. 1962. Three sibling species of *Alectoris* partridges. Ibis 104:353-367.

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

WILLIAMS, D. E., AND R. A. REISFELD. 1964. Disc electrophoresis in polyacrylamide gels: Extension to new conditions of pH and buffers. Ann. N.Y. Acad. Sci. 121:373-381.

WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II: Local genetic differentiation in the Papayo. Am. J. Hum. Genet. 22:24-29.

WRIGHT, S. 1978. Evolution and genetics of populations. Vol. 4, Variability within and among natural populations. Univ. Chicago Press, Chicago.

YANG, S. Y., AND J. L. PATTON. 1981. Genic variability and differentiation in the Galapagos finches. Auk 98:230-242.

ZINK, R. M., D. F. LOTT, AND D. W. ANDERSON. 1987. Genetic variation, population structure, and evolution of California Quail. Condor 89:395-405.

ZINK, R. M. 1989. The study of geographic variation. Auk 106:157-160.

ZINK, R. M., AND J. C. AVISE. 1990. Patterns of mitochondrial DNA and allozyme evolution in the avian genus *Ammodramus*. Syst. Zool. 39:148-161.