

The role of history vs. demography in shaping genetic population structure across an ecotone: chukar partridges (*Alectoris chukar*) as a case study

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ABSTRACT

Recent advances in molecular ecology allow better assessment of the role of ecological and evolutionary processes in determining the dynamics of genetic diversity in natural populations. The chukar partridge (*Alectoris chukar*) is abundant and continuously distributed in the mesic Mediterranean regions of Israel, becoming scarce and patchily distributed in the arid Negev Desert. This demographic transition occurs across a main environmental transition, the Northern Negev ecotone. Populations of chukars along this gradient have been shown to differ from other populations in morphology, bilateral asymmetry, and in allozyme variation. The aim of this study is to infer whether historical (i.e. secondary contact of formerly vicariant populations) or recurrent demographic events (i.e. restriction to gene flow across the ecotone) have shaped the observed patterns of genetic diversity in chukar populations in Israel. We analysed mtDNA control-region sequences in 216 chukars collected from 28 localities (five distinct geographical regions). Results show that (1) average haplotype diversity is high ($h = 0.93$) while haplotype divergence is low ($\pi = 0.8\%$), suggesting that extant populations diversified recently; (2) mtDNA gene diversity does not significantly differ across the distribution range; and (3) haplotype distribution differs among the sampling locations (AMOVA; $P < 0.00031$). Mantel test and autocorrelation analyses indicate that isolation-by-distance may explain the geographical partitioning of genetic diversity. These patterns have likely been produced by complex dynamics between past fragmentation of northern vs. intermediate and southern populations. Late Pleistocene climatic changes, fostering cycles of range contraction and expansion, may have led to genetic differentiation in allopatry, followed by restricted gene flow across secondary contact zones.

Keywords

Alectoris chukar, chukar partridge, ecotone, environmental transition, mtDNA control-region.

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INTRODUCTION

Complex interplays between historical and ongoing evolutionary processes have generated the patterns of geographical diversification that are currently observed in plant and animal populations (Avice, 2000). Assessing the role of recurrent ecological and past evolutionary processes in determining the dynamics of genetic diversity in natural populations is a central challenge facing molecular ecologists and evolutionary biologists (Templeton, 1998). Novel genetic (Emerson *et al.*, 2001) and morphometric approaches are being applied to describe the patterns of geographical population structuring (Storfer, 1996; Roy & Foote, 1997; Sunnucks, 2000). However, separating the effects of

recurrent ecological vs. historical determinants of genetic diversity is challenging, especially in continuously distributed populations that are connected by high dispersal rates, yet are significantly differentiated across their range (Holsinger & Mason-Gamer, 1996; Hutchison & Templeton, 1999; Sork *et al.*, 1999).

Geographical diversification and historical dynamics of populations

During the past 2 million years, the Northern Hemisphere has experienced deep climatic oscillations, which cyclically changed landscapes and habitats in many areas, including Eurasia, the

Mediterranean Basin, and North Africa (Webb III & Bartlein, 1992). Many plant and animal species reacted to these climatic changes by demographic fluctuations and geographical shifts, including range contractions and expansions (FAUNMAP Working Group, 1996; Dynesius & Jansson, 2000). Extant species that are continuously distributed in mesic habitats survived in fragmented refugial patches during the Pleistocene glacials in Europe (Hewitt, 2000). On the other hand, those species that are more adapted to colder or drier habitats, and that were continuously distributed during glacials, now survive as fragmented relics (Bos & Sites, 2001). Thus, current ecological factors alone may not fully explain the present geographical distributions of species and populations.

Historical events left detectable marks also on the genetic structure of populations (Hewitt, 2000). Range expansion following periods of allopatric fragmentation sometimes generated secondary contact zones between conspecific populations. Multiple coincident contact zones involving different species have been described as 'suture zones', as discussed by Taberlet *et al.* (1998). In such areas, the genetic discontinuities often observed across populations that originated by secondary contacts cannot be explained by current demographic or ecological factors alone (Barton & Hewitt, 1989). However, distinguishing genetic discontinuities that originated by secondary contacts or by selection across environmental clines may be difficult, particularly in populations continuously distributed across sharp environmental transitions, such as the chukar (*Alectoris chukar*) in Israel. In these cases, a challenge is to identify geographical diversity patterns resulting from primary differentiation (i.e. isolation-by-distance as a by-product of natural selection and restricted gene flow across heterogeneous habitats), or from secondary contact.

The climatic and ecological gradient in Israel

Israel is located at the crossroad of three main biogeographical regions (Africa, Asia, and Europe) and spans a wide variety of climatic and environmental conditions along relatively short geographical distances (Yom-Tov & Tchernov, 1988; Blondel & Aronson, 1999). A steep transition from the mesic Mediterranean climate of northern and central Israel to the arid-hot Negev Desert occurs across the Northern Negev ecotone, a narrow belt less than 60 km wide where average annual rainfall sharply decreases from over 450 mm to less than 150 mm (Fig. 1). This is an area of transition between the Mediterranean, steppic Irano-Turanian, and desertic Saharo-Arabian-Sudanese phytogeographical regions (Danin & Plitman, 1987).

The structure of biotic communities and the spatial patterns of genetic variability in Israel have been partially explained by differential selective pressure associated to contemporary ecological conditions. Nevo and collaborators described concordant trends of decreasing gene diversity in northern vs. southern populations of many plant and animal species (Nevo, 1988). The niche-width hypothesis (Van Valen, 1965; Nevo, 1978) was invoked as the main deterministic force to maintain different levels of within-population diversity. However, the genetic structure of ecologically subdivided populations could

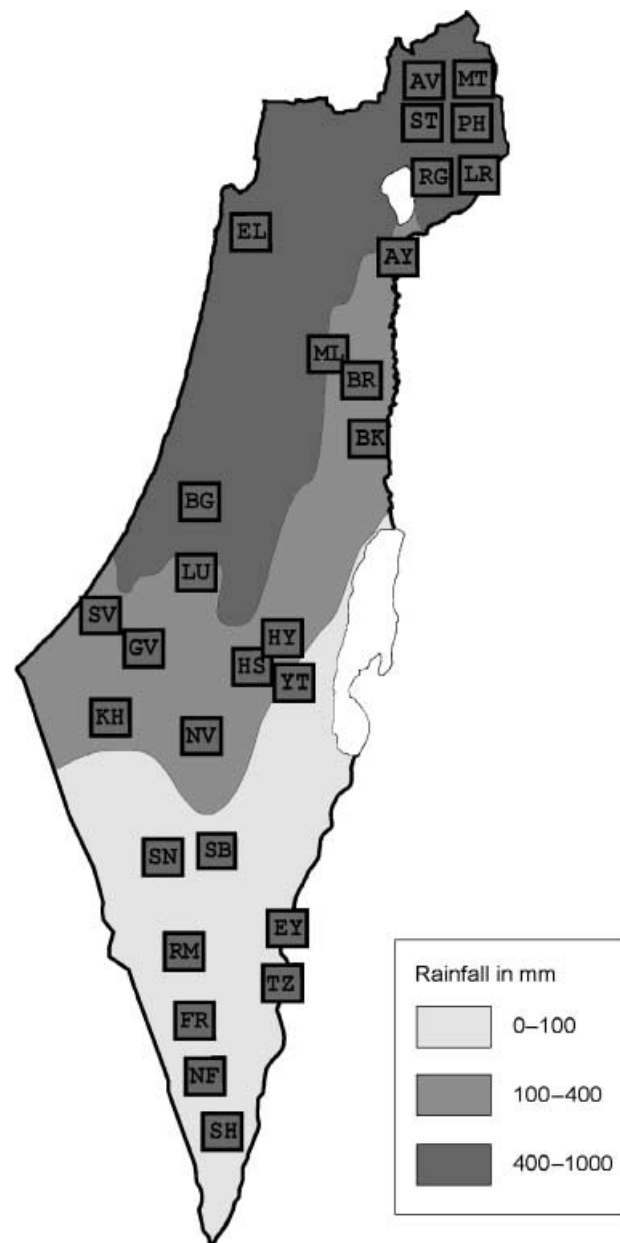


Figure 1 Sampling localities of *Alectoris chukar* in Israel. Population acronyms correspond with localities listed in Table 1. Large-scale vegetation regions and corresponding average long-term rainfall are indicated (see also Table 1).

also be explained by restrictions to gene flow independent from selection, by metapopulation dynamics, or by historical factors, mainly secondary contact of formerly vicariant populations (Comes & Abbott, 1999).

In this study, we aim to infer the role of historical biogeography and current ecology in shaping the patterns of genetic diversity in chukar populations distributed across a sharp environmental transition in Israel. We analyse the geographical distribution of nucleotide sequences in the non-coding mitochondrial DNA control-region (mtDNA CR). Phylogenetic relationships among mtDNA haplotypes are then used to infer whether the observed

phylogeographical patterns are explained by isolation-by-distance (representing ecological factors), by past allopatric fragmentation and range expansion (representing historical factors), or by an interaction of both factors.

MATERIALS AND METHODS

The chukar partridge

The chukar partridge (*A. chukar*; Aves, Phasianidae) is distributed in Israel across a sharp environmental cline, spanning the Mediterranean regions in the north, where mean annual rainfall runs between 450 mm and 1000 mm, and the extremely arid areas in the Negev Desert, only a few hundreds of kilometres away, where mean annual rainfall decreases to less than 50 mm (Fig. 1). An especially steep part of the rainfall gradient occurs in the Northern Negev ecotone region. In this transition area between Mediterranean and desert ecosystems, the annual rainfall decreases threefold within a belt of less than 60 km and its spatiotemporal variability largely increases (Bitan & Rubín, 1991). Chukars do not possess physiological adaptations to heat stress, and reach the limit of their global distribution range in the arid areas of the southern Negev (Carmi-Winkler *et al.*,

1987; Shirihai, 1996). From the ecotone towards the desert, the chukar distribution becomes patchy and the populations become small and non-continuous (Shirihai, 1996; Kark *et al.*, 1999). The chukar is sedentary across its Eurasian range and does not exhibit known latitudinal or altitudinal migration (Shirihai, 1996). Chukars in Israel and in the Sinai Desert vary phenotypically (Nissani, 1974; Shirihai, 1996) and genetically (Randi & Alkon, 1994; Kark *et al.*, 1999). Two distinct subspecies have been proposed: *Alectoris chukar cypriotes*, distributed mostly in the Mediterranean region, and *Alectoris chukar sinaica* in the Sinai Desert (Nissani, 1974).

Study samples

A total of 216 *A. chukar* were collected between September 1995 and early February 1996 from 28 localities distributed across the rainfall gradient from the northern Golan Heights to the southern Negev Desert in Israel (Table 1; Fig. 1). Birds were sampled by Nature and Parks Authority rangers and authorized hunters during the non-breeding season when organized in stable coveys that maintain relatively fixed home ranges (Alkon, 1974). In most cases, one bird, rarely two, was sampled from each covey. To obtain larger sample sizes for statistical analyses, sampling

Table 1 Summary description of *Alectoris chukar* collection sites along a north–south gradient in Israel. Locations are mapped in Fig. 1. Distances are in km from Avital

Region	Population	Code	<i>n</i>	Distances	Environment
Golan Heights	Avital	AV	9	0	Orchards
	Mahfi Tel	MT	13	3	Mediterranean maquis
	Pazra Hill	PH	9	12	Mediterranean maquis
	Shifon Tel	ST	5	6	Orchards
	Lawiya	LR	12	31	Open oak forest
Mediterranean	Ramot	RG	5	32	Orchards
	Elyakim	EL	5	87	Mediterranean
	Ben-Gurion Airport	BG	7	148	Ruderal vegetation
North Ecotone	Luzit	LU	9	181	Mediterranean
	Bezek	BR	5	80	Natural
	Ashdot Ya'akov	AY	4	54	Orchards
South Ecotone	Malkishua	ML	4	83	Natural
	Gvaraam orchards	SV	10	200	Orchards
	Gvaraam	GV	6	204	Steppe
	Hirbet Yatir	HY	12	209	Steppe
	Hirbet Sira	HS	7	213	Steppe
	Yatir	YT	1	214	Orchards
	Khatzerim	KH	3	232	Steppe
Nevatim	NV	12	222	Steppe	
Negev Desert	Negev Highlands	SN	17	260	Desert
	Sede Boqer	SB	15	265	Orchards
	Ein Yahav	EY	5	284	Orchards
	Ramon	RM	15	283	Desert
	Tzofar	TZ	2	290	Orchards
	Northern Faran	NF	3	299	Desert
	Faran	FR	12	317	Desert
	Shittim	SH	3	334	Orchards

locations were grouped into five predetermined ecogeographical regions, which were delimited according to the prevalent local ecological conditions (climate and habitat similarity; see Table 1): the Golan Heights ($n = 53$), the Mediterranean coastal strip ($n = 21$), the northern part of the Negev ecotone ($n = 19$), the southern part of the Negev ecotone ($n = 51$), and the Negev Desert ($n = 72$; Table 1). Twenty chukars from Gansu region in China were used as an outgroup for phylogenetic analyses.

DNA extraction, amplification and sequencing

Total DNA was extracted from liver samples, stored in 100% ethanol, using guanidinium thiocyanate and diatomaceous silica particles (Gerloff *et al.*, 1995). The entire mtDNA CR was polymerase chain reaction amplified. About 450 nucleotides of the left side of the CR, including the hypervariable domain CR-IA (Randi & Lucchini, 1998), were sequenced using the PRISM Dye-Terminator chemistry in an ABI 373XL automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned and manually edited with SE-AL 1.0 (A. Rambaut, <http://evolve.zoo.ox.ac.uk/Se-AL/Se-AL.html>). Unique haplotypes were identified using COLLAPSE 1.0 (D. Posada, http://bioag.byu.edu/zoology/crandall_lab/programs.htm).

Analyses of genetic diversity and population structure

We used the software ARLEQUIN 2.0b2 (<http://anthropologie.unige.ch/arlequin>) to compute estimates of genetic diversity, molecular variance (AMOVA; Excoffier *et al.*, 1992), values of the fixation indices (Weir & Cockerham, 1984) among populations (F_{sc}) and among regions (F_{ct}), an exact test of differentiation based on haplotype frequencies (Raymond & Rousset, 1995; Goudet *et al.*, 1996), Tajima's (1989) D -test of selective neutrality, and to test the hypothesis that populations diversified through isolation-by-distance (Rousset, 1996). The pattern of geographical population structuring was described by autocorrelation analysis using AIDA, a software that computes values of the I and c indices, analogous to Moran's i and Geary's c , and incorporates information on genetic distances among the haplotypes (Bertorelle & Barbujani, 1995). Autocorrelation indices were calculated independently in a number of distance classes with defined limits or of equal size, with the number of sequence comparisons in each class being approximately equal. Due to the uneven spatial distribution of the samples, we used distance classes of pre-defined size (results did not change using classes of equal size). Discontinuities in the distribution of genetic diversity among regions were assessed by a hierarchical analysis of variance, as implemented in the NUCLEODIV procedure (Holsinger & Mason-Gamer, 1996).

Phylogenetic analysis of mtDNA sequences

The phylogenetic relationships among the CR haplotypes were reconstructed using PAUP* 4.0b2a (Swofford, 1998), and (1) heuristic maximum-parsimony (MP) procedure, with exclusion of all uninformative nucleotide positions, unordered and equally

weighted characters, random haplotype additions with 10 replications per run, multiple minimal trees swapped by tree bisection and reconnection, and collapsed zero lengths branches; and (2) the neighbour-joining algorithm (NJ; Saitou & Nei, 1987), with the best-fit maximum-likelihood (ML) DNA distance measure. The best-fit ML substitution model was selected by likelihood ratio test among a suite of models of increasing complexity (Huelsenbeck & Crandall, 1997), using PAUP* and MODELTEST (Posada & Crandall, 1998). Multiple MP and ML trees were collapsed to obtain strict and 50% majority-rule consensus trees. Support to the internodes was obtained by 1000 bootstrap resampling.

RESULTS

Sequence description and population variability

We sequenced 444 nucleotides of the mtDNA CR, mapping at positions 1-444 of the CR of *Alectoris* (Randi & Lucchini, 1998), in 216 samples of chukars collected from 28 locations in Israel (Table 1). The alignment showed 37 different haplotypes (Table 2), defined by 24 polymorphic sites. The average haplotype diversity was $h = 0.93$, with the lowest h -values showed by the Golan and Negev Desert populations ($h = 0.90$), and the highest one ($h = 0.93$) by the Mediterranean populations (Table 3). All these values did not significantly differ from each other (Student's t -test, $P < 0.05$). The average pairwise divergence between haplotypes was 3.5 ± 1.2 mutations (range 1-9 mutations). Nucleotide diversity in the populations was low (average $\pi = 0.008$), with the lowest value found in the north ecotone populations ($\pi = 0.006$), and the highest found in the Golan populations ($\pi = 0.008$; these differences were not significant; Student's t -test, $P < 0.05$). These mtDNA CR sequences evolved neutrally (Tajima's $D = -0.355$, $P > 0.10$).

The distribution of the haplotypes in the five geographical regions (Table 2) showed a composite pattern. There were eight common haplotypes (with relative frequency ranging from 3.7% to 15.7%), which were widespread and shared by populations in all five regions (haplotypes 11, 5, 12, and 17), or in four (haplotypes 23, 22, 31, and 18) of the five regions. Five haplotypes were present at frequency equal to or lower than 3.2% in two regions, and there were 24 'private' haplotypes (with frequency lower than 1%), each restricted to only one of the five regions. Haplotype frequencies were significantly different in the two geographically most distant Golan and Negev Desert regions (Fig. 2), which shared only four common haplotypes (5, 11, 12, and 17), while nine and five haplotypes were private in these two regions, respectively (Table 2).

The AMOVA indicated that 84.3% of total genetic variability was distributed within populations, 6.6% among populations (within regions), and 9.0% among regions. Values of the fixation indices among populations (within regions) and among regions were $F_{sc} = 0.073$ (the probability to obtain random values equal or larger than observed was $P = 0.00012$; 16,000 permutations), and $F_{ct} = 0.090$ ($P = 0.00031$; 16,000 permutations), respectively. The exact test indicated that the probability of non-differentiation

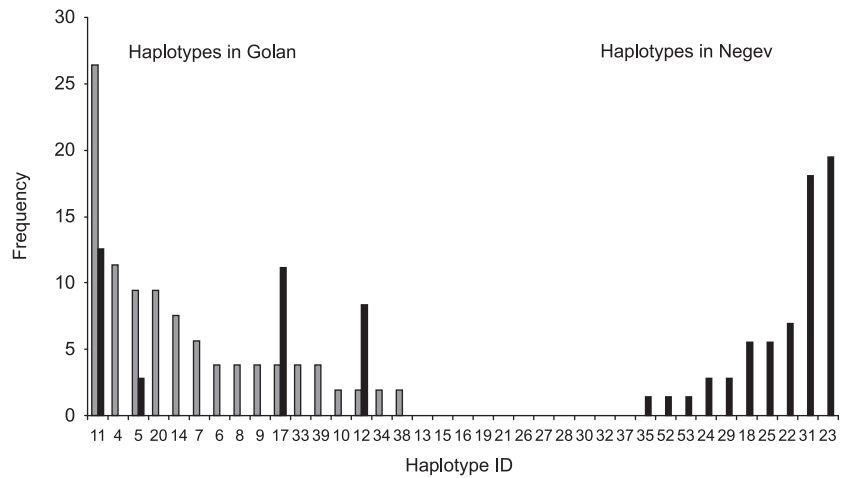


Figure 2 Frequency distributions of chukar mtDNA haplotypes in northern Golan Heights and southern Negev Desert populations. Haplotype ID numbers are indicated as in Table 2.

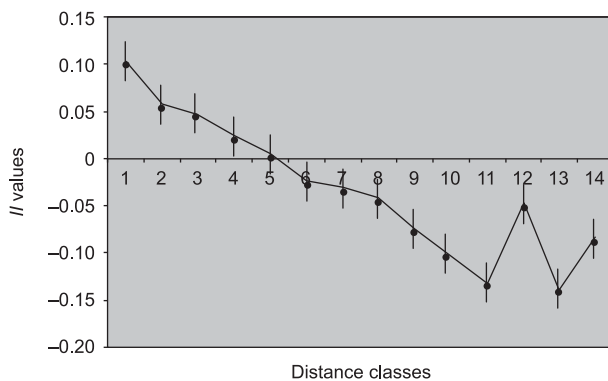


Figure 3 AIDA autocorrelogram (Bertorelle & Barbujani, 1995), plotting *I* values against 14 distance classes (dots). Standard errors of *I* values are indicated by vertical bars.

among population was $P < 0.00001$ (10,000 Markov steps). These findings revealed that the geographical distribution of the mtDNA haplotypes was significantly different among chukar populations within regions and among regions.

Geographical population structure

Isolation-by-distance was tested by a Mantel test, which showed a matrix correlation coefficient $r = 0.74$ ($P = 0.065$; 10,000 permutations). Thus, there was a weak, statistically non-significant, isolation-by-distance effect. The autocorrelograms, computed by AIDA using all 28 populations and eight to 14 distance classes (Fig. 3), did not show the monotonous and regular pattern of decreasing values of autocorrelation indices that is expected in the case of isolation-by-distance. In fact, the greatest distance classes showed a reversal of the main autocorrelation trend, suggesting that populations located more than *c.* 200 km apart were equally or less negatively autocorrelated than populations at intermediate distance classes. The autocorrelation trends did not change for different distance classes.

Discontinuities in the distribution of genetic diversity between populations were assessed using the NUCLEODIV procedure



Figure 4 Hierarchical partitioning of genetic diversity within (values in brackets near the internodes) and between populations (values in brackets following the population names), computed using the NUCLEODIV procedure (Holsinger & Mason-Gamer, 1996). Significance of the between population differences are indicated. Chinese chukars (China) are used as outgroups.

(Fig. 4). The hierarchical partitioning of genetic diversity (both with and without the Chinese chukar outgroup) within and among regions indicated that Negev Desert and Golan populations were significantly differentiated from Mediterranean and ecotonal populations ($P < 0.0001$). On the other hand, the Mediterranean, and the southern and the northern ecotone populations did not significantly differ from one another. The geographically most distant populations were also the genetically most differentiated. However, the Mantel test and autocorrelation analyses suggest that isolation-by-distance cannot fully explain the pattern of geographical differentiation.

Phylogenetic structure of the mtDNA haplotypes

The strict-consensus MP tree (based on 60,000 equally parsimonious trees obtained from 14 parsimony informative characters; length $L = 35$; consistency index $CI = 0.40$, retention index $RI = 0.77$) showed two main clades, A and B, which were internally unresolved (Fig. 5a). A similar topology was obtained by NJ analysis using the best-fit substitution model TN93 + I ($I = 90\%$ invariable sites), as indicated by MODELTEST (not shown). MP and NJ trees, rooted using homologous mtDNA CR sequences from Chinese chukars, showed that haplotypes from Israeli chukars form a monophyletic clade, the root being intermediate between clades A and B (Fig. 5a).

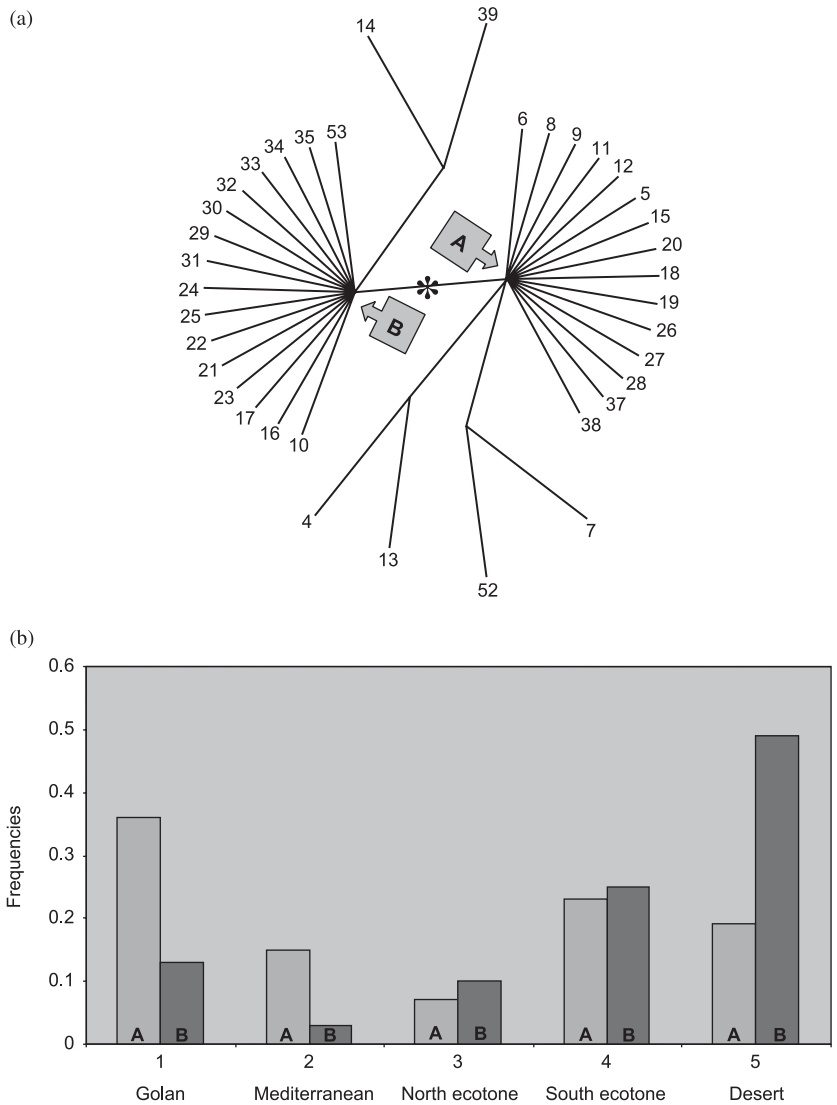


Figure 5 (a) Maximum-parsimony tree of chukar mtDNA haplotypes computed using PAUP* (Swofford, 1998); and (b) frequency distribution of clade A and clade B haplotypes in the chukar populations in Israel. The asterisk indicates the position of the root of the tree, obtained using Chinese chukar outgroups.

Haplotypes in clades A and B were distributed with different frequencies in the five geographical regions (Fig. 5b). Populations in the Golan included mainly clade A haplotypes, whereas populations in the Negev Desert included mainly clade B haplotypes. Populations in the Mediterranean region included mainly clade A haplotypes, whereas the composition of the ecotonal populations was equal. Clade A included five of the nine private Golan haplotypes, plus widespread and ecotonal haplotypes. Clade B included four of the five private Negev Desert haplotypes, plus widespread and ecotonal haplotypes. However, clade A included also the private Negev Desert haplotype 52, and clade B included the private Golan haplotypes 10, 33, and 34.

DISCUSSION

Patterns and processes of genetic diversification

This study reveals that chukars in Israel have high mtDNA diversity but low haplotype divergence (Table 3; for comparison with other wild galliform populations see Lucchini & Randi,

1998; Holder *et al.*, 1999). Despite the high substitution rate of the hypervariable mtDNA CR domain in *Alectoris* (Randi & Lucchini, 1998), these haplotypes showed substitutions at 0.8% nucleotides, on average, suggesting that they originated recently. The phylogenetic trees were star-shaped, showing a few common haplotypes, presumably ancestral, at the centre of the topologies. The coalescent theory indicates that star-shaped topologies likely result from recent and sudden population expansion (Emerson *et al.*, 2001). However, about 65% of the haplotypes were private, i.e. they were found only in one of the five geographical regions sampled, suggesting that dispersal and gene flow might have been restricted during recent population expansion.

Haplotype frequency distributions and the AMOVA indicated that northern (Golan) and southern (Negev) populations were significantly differentiated. The presence of widespread haplotypes in both clades A and B suggests either that populations are panmictic and interconnected by gene flow across the ecotonal region, or that they did not diverge enough to reach a state of reciprocal monophyly and still shared ancestral haplotypes. Thus, the causes of the observed population structure cannot be

inferred unambiguously either by the geographical distributions of haplotype frequencies or by their genealogical relationships. The haplotype trees were unresolved and a parsimony network was widely reticulated (not shown), thus preventing the possibility to perform nested clade analyses (Templeton, 1998). The spatial structure does not fully fit a simple isolation-by-distance model. Isolation-by-distance is expected in populations at equilibrium between migration and drift, when the haplotype frequency distribution is the result of current demographic processes rather than historical associations (Hutchison & Templeton, 1999). Weak isolation-by-distance and the autocorrelation patterns suggest that chukar populations were not in genetic equilibrium in the study areas.

The role of natural selection and demography

Chukars in Israel show geographical variation in allozyme loci (Randi & Alkon, 1994; Kark *et al.*, 1999), bilateral asymmetry (Kark, 2001; Kark *et al.*, 2001), and in multiple morphological traits (Kark, 1999; Kark *et al.*, 2002). Allozyme heterozygosity is significantly lower in the Golan and Negev populations, and higher in populations sampled from the ecotonal areas (S. Kark *et al.*, unpublished data). The origin of the pattern of allozyme diversity is not obvious. At least some of the allozyme loci could be, directly or indirectly, selected. Genetic diversity within a population could be maintained by disruptive selection in the patchwork of variable habitats across the ecotone (S. Kark *et al.*, unpublished data). Alternatively, secondary contacts of formerly allopatric populations across the ecotone are expected to increase the heterozygosity (Randi & Alkon, 1994).

Kark (2001) provides evidence for changes in bilateral asymmetry in chukar across short geographical distances in Israel. Substantial theoretical, experimental, and observational work suggests that the estimation of developmental instability using bilateral asymmetry may serve as a tool for assessing the degree to which individuals were able to buffer their development in the face of stress (e.g. Leary & Allendorf, 1989; reviewed in Lens *et al.*, 2002). Kark (2001) examined the length of the third toe in 11 chukar populations (see Kark, 2001 for more details on measurement and traits). Three principal types of bilateral asymmetries in morphological traits (i.e. deviation from perfect symmetry between the right and the left sides of an organism's body) have been described in the literature. These include fluctuating asymmetry (FA), directional asymmetry (DA), and antisymmetry (Van Valen, 1962; Palmer & Strobeck, 1986). The three differ in the distribution of the signed difference between the right and the left (R-L) sides of a trait in a population. Fluctuating asymmetry is defined (based on Ludwig, 1932) as the small random non-directional deviations from perfect bilateral symmetry in morphological traits in which symmetry is the normal state. In traits exhibiting FA, signed R-L values in the population are predicted to be normally distributed around a mean of zero. In traits showing DA, there is a consistent bias towards over development of one specific side relative to the other, and the R-L distribution shows a non-zero mean (Van Valen, 1962; Palmer & Strobeck, 1986). Antisymmetry (handedness) is

similar to DA in the sense that one side is usually larger than the other, yet the population shows a broad-peaked (platykurtic) or a bimodal distribution of signed R-L around a mean of zero (Palmer & Strobeck, 1986). In the chukar study, Kark (2001) found that asymmetry significantly increases from Mediterranean to desert populations. The proportion of symmetrical individuals in the population decreased across the gradient towards the range periphery, and the least symmetrical individuals were sampled from the arid periphery of the range. Transition from fluctuating asymmetry to antisymmetry and to directional asymmetry can reflect increasing environmental stress and severe developmental instability rather than genetic variation. Morphological diversity at multiple-correlated traits was higher in the population of the Northern Negev ecotone compared to all the other populations from both the Mediterranean and the arid peripheral areas of the range (Kark, 1999). Morphometric studies usually cannot distinguish the genetic and environmental components of phenotypic diversity. However, at least some of the traits analysed are correlated with fitness, flight, movement, and feeding ecology, and might be heritable and, thus, selected in birds (Boag & van Noordwijk, 1987; Smith *et al.*, 1997).

Rates of gene flow between the Golan and Negev populations are $Nm = 9-12$ and $Nm = 2.6$, as estimated using allozymes and mtDNA, respectively. Thus, the observed phenotypic and genetic differences among chukar populations are maintained despite substantial gene flow. Genetic divergence in adaptive quantitative traits might in fact be maintained if natural selection overrides gene flow, as suggested by the divergent-with-gene-flow model of speciation (Rice & Hostert, 1993). However, it is difficult to envision a generalized kind of selection in ecologically subdivided populations that can explain concordant patterns of diversification at multiple biochemical, molecular, and morphological traits (Nevo *et al.*, 1999; Turpeinen *et al.*, 2001). Those traits are expected to have different adaptive values, ranging from complete neutrality to strong stabilizing selection, and should evolve independently (Comes & Abbott, 1999; Lynch *et al.*, 1999).

The genetic structure of ecologically subdivided populations could also be explained by restrictions to gene flow, independent of selection. For example, gene flow could be lower in arid compared with more mesic habitats due to the higher level of population subdivision and limited dispersal across a matrix of unsuitable habitats. However, studies designed to test these models showed contrasting results in a number of species (Comes & Abbott, 1999; references therein). In this study, both the NUCLEODIV procedure and the pairwise tests of sample differentiation suggested that Negev Desert and Golan populations were significantly differentiated, whereas Mediterranean, ecotonal, and desert populations were not significantly differentiated. Pairwise estimates of Nm also showed that gene flow was high between northern populations (Nm between Golan and Mediterranean = 6.11) as well as between southern populations (Nm between south ecotone and Negev Desert = 13.90). Nm was lower across the ecotone (Nm between north and south ecotone = 2.10). Thus, rates of gene flow in chukars were not lower in the arid compared to the Mediterranean more mesic environments.

Climatic history

The simplest model that can explain the phylogeographical patterns observed in chukars is a combination of past fragmentation and isolation in allopatry with ongoing restricted gene flow. Gene flow among expanding populations is probably constrained by selective barriers across the ecotone, which may filter gene introgression, preventing a complete population admixture and maintaining isolation-by-distance.

Population fragmentation is the likely outcome of past climate and habitat changes (Hewitt, 2000). The fossil pollen record suggests that the Mediterranean climate has been in phase with the global climate during the Late Quaternary (Rossignol-Strick, 1998). Recurrent climatic fluctuations affected temperature and rainfall regimes in the Near East, produced changes in vegetation, and repeatedly shifted the desert boundaries in the Eastern Mediterranean regions (Magaritz & Goodfriend, 1987). Pollen data from interglacial periods (125, 193, and 240 kyr BP) indicate prevalence of mixed deciduous oak (*Quercus*) forest in the Levant (Miller, 1997; Rossignol-Strick, 1998). In contrast, the pollen record from the glacial (175 and 225 kyr BP) shows the prevalence of sage-brush (*Artemisia*) semidesert, suggesting drier climates and winter lower temperatures than present (Miller, 1997; Rossignol-Strick, 1998).

During the last glacial maximum (LGM; 23–15 kyr BP) there were no trees, but a prevalence of *Artemisia* and Chenopodiaceae semidesert vegetation in the Eastern Mediterranean regions, similar to vegetation of the Asian steppes where chukar populations are living today. The LGM was followed by a deglaciation pulse (15–11 kyr BP; Termination Ia), when pine trees and mixed oak forests spread again. The Younger Dryas (11–10 kyr BP) was dry with no forests and abundant Chenopodiaceae steppes. In the Holocene (after 10 kyr BP), pine trees were rapidly replaced by mixed oak forests (with other deciduous trees: *Tilia*, *Corylus*, *Fraxinus*, *Ulmus*), similar to the present forests at mid-high latitude of oceanic Europe, and in the cool and wet south coast of the Black Sea (as in the Amanus Mountain in south-east Turkey), where chukars are not currently distributed (Cramp & Simmons, 1980).

The ecological niche of chukars is wide and does not allow us to infer simple correlations between habitat variation and population dynamics. Chukars generally inhabit mesic and semi-arid areas with large populations in Mediterranean and steppe regions in Israel and in the Near East (Shirihai, 1996). In the desert, chukar density decreases, distribution becomes discontinuous and local populations are small and patchy (Shirihai, 1996). Chukar populations likely contracted during wet interglacials and early Holocene when *Quercus* forests expanded, while populations expanded during arid glacial when *Artemisia* semidesert expanded. The most recent chukar population expansions could have occurred during the Younger Dryas and during the 'pistachio tree' phase (9–6 kyr BP), a climate optimum characterized by expansion of the Mediterranean maquis and contraction of the temperate deciduous forest (Rossignol-Strick, 1998). A combination of vastly increased human activity, including grazing, agriculture, forest clearing

during the last 4–6 kyr, modern agriculture, and irrigation in the past 150 years had likely determined the ecological conditions for the chukar expansion in the Levant.

The chukar populations currently distributed across the ecotone are likely to be the remnants of the most recent population expansions. Present-day environmental clines could contribute to maintain the genetic diversification by restricted gene flow across the transition zone. Thus, the observed genetic structure of chukar populations in Israel has likely been determined by a combination of complex historical processes (past fragmentation in allopatry and range expansion) and demographic processes (long- and short-range dispersal across the environmental cline).

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