Genetic structure of Black Grouse (Tetrao tetrix) populations of the French Alps

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Original article

Genetic structure of Black Grouse (*Tetrao tetrix*) populations of the French Alps

Alain CAIZERGUES[a,b,*], Sophie DUBOIS[c], Guénaëlle MONDOR[c], Anne LOISEAU[c], Laurence N. ELLISON[a], Jean-Yves RASPLUS[c]

[a] Office national de la chasse et de la faune sauvage, Faune de montagne, 8 impasse Champ Fila, 38320 Eybens, France
[b] Centre national de la recherche scientifique, Centre d'écologie fonctionnelle et évolutive, 1919 route de Mende, 34293 Montpellier Cedex 5, France
[c] Institut national de la recherche agronomique, Centre de biologie et de génétique des populations, 488 rue Croix de Lavit, 34090 Montpellier, France

Abstract – Allelic variation at eight microsatellite loci was used to assess the levels of genetic differentiation between seven natural populations of black grouse (*Tetrao tetrix*) in the French Alps spaced along a 250 km south-north transect. Whatever the population or locus, genotype frequencies did not deviate significantly from expected Hardy-Weinberg frequencies and no significant between-locus linkage desequilibrium was detected. Observed levels of genotypic variation were statistically significant with maximum Fst values reaching 10% for the most distant populations (250 km). An isolation-by-distance effect was detected suggesting, as expected from data on marked birds, that black grouse populations in the French Alps are interconnected by dispersal.

birds / isolation-by-distance / microsatellites / population structure / Tetraonidae

Résumé – Structuration génétique des populations de tétras-lyre (*Tetrao tetrix*) des Alpes françaises. La structuration génétique de sept populations naturelles de Tétras-lyre selon un transect allant de la Haute-Savoie aux Alpes maritimes a été étudiée au moyen de huit locus microsatellites. Quelles que soient les populations et les locus, les fréquences génotypiques ne s'écartent pas significativement des proportions attendues sous l'hypothèse de Hardy-Weinberg et aucun déséquilibre de liaison significatif n'a été détecté. À l'exception des populations géographiquement les plus proches (*i.e.*, séparées d'une vingtaine de km ou moins) les différenciations génotypiques observées sont statistiquement significatives. Les valeurs de F_{st} maximales obtenues entre les populations les plus éloignées (jusqu'à 250 km) avoisinent 10 %. La

* Correspondence and reprints
E-mail: caizerg@hotmail.com

différenciation génotypique des populations de tétras-lyre suit clairement, à l'échelle des Alpes françaises, un modèle d'isolement par la distance. Selon ces résultats qui sont congruents avec les données obtenues au moyen de méthodes directes, les populations de Tétras-lyre des Alpes françaises seraient interconnectées.

***avis* / isolement par la distance / microsatellites / structuration génétique / Tétraonidés**

1. INTRODUCTION

Data on both demographic parameters and social structure are of key importance for the conservation of natural animal populations [3]. Two main approaches are currently used in demographic and/or behavioural studies: i) the direct approach which uses census data and/or information gathered on individually marked animals (capture-recapture, radio-tracking...), and ii) the indirect approach, using molecular markers to infer parentage and gene flow.

Although direct and indirect methods are often considered as alternatives, they both have advantages and drawbacks for estimating parameters of interest in conservation/management. The direct approach is efficient for estimating most demographic parameters [9], but can rarely be applied on a suprapopulational scale [15]. In contrast, thanks to the possibility of obtaining amplifications with minute quantities of template DNA (collected from hairs, feathers, faeces, soft tissues...), indirect methods facilitate large-scale studies. Thus indirect methods are especially suitable for the assessment of spatial structure of mobile, wide-ranging organisms such as birds [2].

The black grouse (*Tetrao tetrix*) is one of the six native game birds inhabiting mountainous areas of France. Although black grouse populations are continuously distributed in the boreal forest from Scandinavia to south-eastern Siberia, those in the western and southern parts of the range have become so fragmented that the populations of the Alps are now probably isolated. Therefore they may be potentially vulnerable to extinction in the long term (see Ref. [27]).

Black grouse populations of the French Alps cannot yet be considered threatened (spring number of males is estimated as 8000–10 000), but a recent decline affecting both the spatial distribution and densities has been documented [4]. For example, of 18 populations where males have been counted during spring display for at least six years, eight are declining, nine are stable and only two are increasing (Observatoire des Galliformes de Montagnes, unpublished data). To investigate the reasons for this decline, a number of studies have been conducted since the mid-seventies. Thanks to these studies, along with those conducted in Fennoscandia, our understanding of habitat requirements, demography [7,17,28] and social structure [13] of black grouse has been greatly enhanced. The information obtained has been useful for improving management of populations and for restoring breeding habitats [5]. However, because

all of these studies were essentially based on direct methods, little is known about the spatial structure of black grouse populations in the Alps. A study has been conducted on continuously distributed populations of central Sweden but over a smaller area and with a very limited set of markers [12]. Thus little is known about the spatial structure of black grouse in general. In the Alps, radio-tracking data along with mapping the distribution of suitable habitats have led us to hypothesise that French populations are interconnected by dispersal [7]. However, because juvenile dispersal is inhibited by such barriers as high rocky ridges [7], confirmation of this hypothesis is needed.

The aim of this study was to assess genetic structure of black grouse populations of the French Alps using microsatellite loci. In particular, we investigated the relationship between genotypic differentiation and geographical distance. Both the absence of genetic structure and an isolation-by-distance effect (increase of genotypic differentiation with geographical distance) would support the idea that the distribution of black grouse populations is continuous. In contrast, a random organisation of genetic structure would suggest the existence of discontinuities (with rocky ridges being, as predicted by radio-tracking, the most likely factor impeding gene flow).

2. MATERIAL AND METHODS

2.1. Sampling of populations and DNA extractions

Tissue samples (liver, muscles, feathers, wings) were collected from males shot in the autumns of 1997 and 1998 at locations shown in Figure 1. In the Queyras, blood samples and growing feathers were taken from living individuals of both sexes captured during summer counts of the same years. Euclidean geographical distances between spatial units sampled ranged between 10 and 250 km (Fig. 1). DNA was extracted using silica columns (QIAamp tissus Kit, Qiagen Inc., standard protocol).

2.2. Microsatellite development

Six microsatellite loci isolated in domestic chicken (ADL146, ADL162, ADL230 [8]; LEI31, LEI0170, LEI0319 [11]) and 10 others specific to red grouse (*Lagopus l. scoticus*) (LLSD2, LLSD3, LLSD4, LLSD5, LLSD6, LLSD9, LLSD10 [20]; LLST1, LLST2, LLST3 [21]) were tested. Of these, only three (LEI319, LLSD4 and LLSD9) gave clearly identifiable and polymorphic PCR products.

Five microsatellites originally isolated in Black Grouse were also used. Of these, three (TTT1, TTT2 and TTD2) were isolated and developed by us (unpublished) and primers for two others (BG2, BG5) were kindly provided

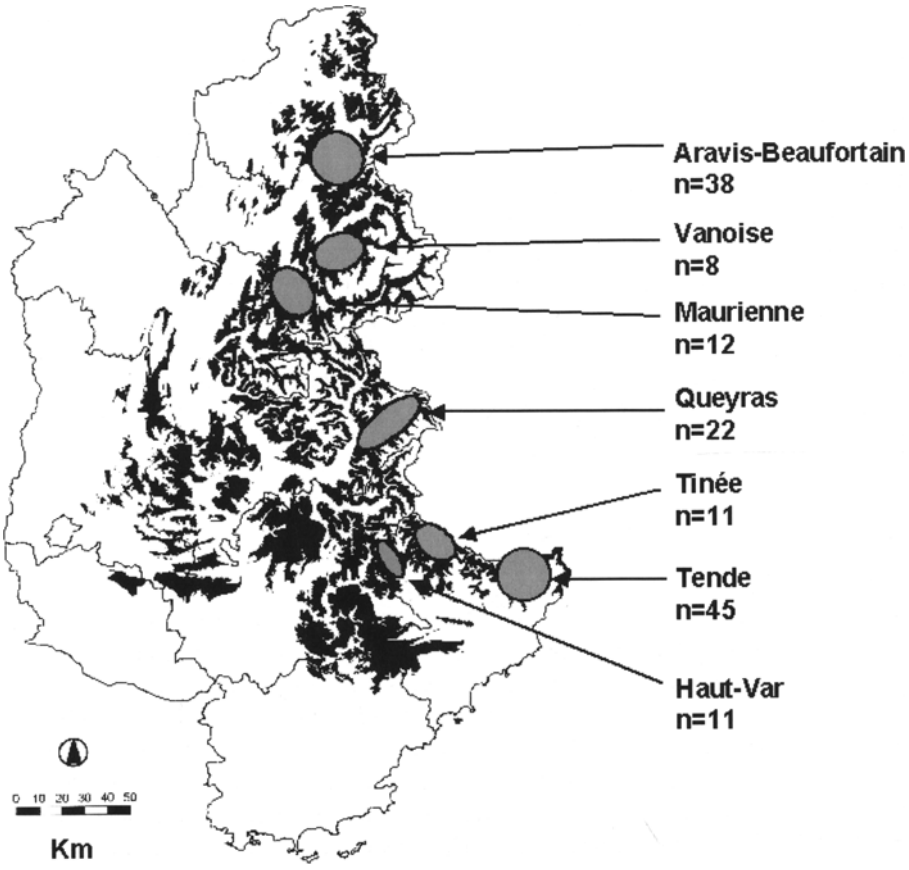

Figure 1. Sampling localities and spatial distribution of habitats favourable to Black Grouse in the French Alps (in black). Sample sizes (n) in parentheses. Map duplicated with permission of the Observatoire des Galliformes de Montagne.

by J. Höglund (Evolutionary Biology Centre - Population Biology, Uppsala University, Sweden, unpublished).

Therefore, the three microsatellites isolated in red grouse and domestic chicken, plus the five from black grouse, gave us eight polymorphic microsatellite loci to characterise our samples.

2.3. DNA amplifications

PCR amplification reactions were performed in a 10 μL final volume using an MJ Research PTC 100 thermal cycler. Each reaction mix contained 1 μL of extraction product, 0.6 μL of 25 mM $MgCl_2$, 0.4 μL of 2.5 mM dNTP, 1 μL of 10x buffer (10 mM Tris-HCl, 50 mM KCl and 0.1% Triton X-100), 0.09 μL

of 5 u/μL Taq polymerase (PROMEGA) and 0.25 μL of each 10 μM primer (forward primer end-labelled with (γ^{33}P-dATP). Length polymorphism was resolved by running PCR products on 6% denaturating polyacrylamide gels and by visualising the fragments by autoradiography. PCR cycles followed a Touch Down [10] procedure. After an initial step of denaturation of 3 min at 94 °C, 10 cycles of PCR were performed, each consisting of 30 s denaturation at 92 °C, 30 s of annealing starting 5 °C above the optimal annealing temperature and dropping by 1 °C per cycle, and finally 30 s of extension at 72 °C. We also included a further 20 cycles consisting of 30 s denaturation at 92 °C, 30 s of annealing 5 °C below the optimal annealing temperature, and 30 s of extension at 72 °C and a 10-min extension at 72 °C following the last annealing step.

Optimal annealing temperatures (for an MJ Research PTC 100 thermal cycler) were 57 °C for LEI319, 55 °C for LLSD4, 56 °C for LLSD9, and 58 °C for TTT1, TTT2 and TTD2.

2.4. Statistical analyses

We computed allele frequencies, observed heterozygosities (Ho) and expected heterozygosities using Nei's (1978) unbiased estimate He [18]. Deviations from Hardy-Weinberg proportions, linkage disequilibrium and between-population genotypic differentiation were tested using exact tests provided in GENEPOP (3.1d version, 1999) [23]. We used Weir and Cockerham [28] F_{st} as estimates of population divergence. For comparison, Slatkin R_{st} (not used in the interpretation of data) are also provided. GENEPOP was also used to test the isolation-by-distance effect with the Mantel procedure [16] by correlating $F_{\mathrm{st}}/(1-F_{\mathrm{st}})$ and the logarithm of the straight-line distance (in kilometres) between pairs of populations [24].

3. RESULTS

A total of 170 samples were collected at the seven locations studied. However, statistical analyses were performed on the 148 individuals genotyped at at least five loci (see Fig. 1).

3.1. Genetic variability

The total number of alleles per microsatellite locus ranged from five for loci LEI319, TTD2 and LLSD9 to 15 for locus LLSD4, and the average number of alleles per locus among populations was 4.8 (Tab. I). This allelic diversity (average number of alleles per locus) ranged from 4.5 for the Vanoise and the Maurienne to 6.4 for Aravis/Beaufortain. Understandably, lowest allelic diversities were generally observed in localities where sample size was small.

However, allelic diversity was higher for Aravis/Beaufortain than for Tende (6.4 *versus* 4.75) even though sample sizes were similar (Tab. I).

Observed mean heterozygosities were similar between localities, ranging from 0.60 for the Maurienne to 0.73 for the Haut-Var and showed no particular trend. Allelic frequencies for the eight loci examined in samples from the seven localities are given in Table I.

3.2. Hardy-Weinberg proportions and linkage disequilibrium

No genotypic linkage disequilibrium could be detected. Only 5 out of 56 population-locus combinations deviated significantly ($P < 0.05$) from Hardy-Weinberg proportions. All of these, which were randomly distributed across loci and populations, became non-significant when the level of significance was adjusted for multiple testing (Bonferroni correction, significance level α set at 0.0009, Tab. I). Similarly, of the 196 exact tests for linkage disequilibrium performed, only 6 were significant and randomly scattered across locus pairs and populations. Of these tests, only one remained significant (LLSD4-BG5 in the Queyras) after Bonferroni correction (significance level α set at 0.0004).

3.3. Genetic differentiation and population structure

Highly significant levels of genotypic variation between populations were detected (mean $F_{st} = 0.06$, Tab. II). Moreover, genotypic differentiation between pairs of populations clearly increased with geographical distance. The correlation between $F_{st}/(1-F_{st})$ and the logarithm of the straight line distance between populations was highly significant ($r = 0.76$, Mantel test; $P < 0.01$, Fig. 2).

4. DISCUSSION

Despite a small data set, our study provides useful insight into the spatial structure of populations of black grouse in the Alps. Comparison of genotypic frequencies reveals statistically significant levels of differentiation between geographic areas. We also show that levels of genotypic differentiation significantly increase with geographical distance. This isolation-by-distance effect implies that populations of the French Alps are connected by substantial gene flow, or that they were connected in the recent past. This finding is consistent with data gathered by direct methods showing that most suitable habitats of black grouse, which are rarely separated by more than 10 km, are potentially interconnected by juvenile dispersal (mean distance of dispersal of juvenile females = 8 km, range 1 to 29 km, mean distance of dispersal of juvenile males 1.5 km, range 0.1 to 8 km). Moreover, although radio-tracking data suggest that dispersing black grouse are apparently reluctant to cross rocky ridges rising above 2500 m [7],

Table I. Distribution of allele frequencies at the eight microsatellite loci in the seven localities sampled. Also given are number and size (relative size for loci LEI319. LLSD4. LLSD9. BG2. BG5 and exact size for loci TTT1, TTT2 and TTD2) of alleles indentified and observed (Ho) and expected (He) heterozygosities, together with associated probabilities (P) of exact tests of Hardy-Weinberg proportions.

Locus LEI319	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		37	8	12	21	10	11	45	
Number of alleles		5	4	4	4	4	4	4	4.14
	0	0.03	0.13	0.08	0.41	0.15	0.50	0.42	
	2	0.23	0.06	0.38	0.07	0.15	0.09	0.07	
	6	0.01	-	-	-	-	-	-	
	8	0.60	0.63	0.46	0.48	0.55	0.36	0.40	
	10	0.14	0.19	0.08	0.05	0.15	0.05	0.11	
Heterozygote Proportion (Ho)		0.65	0.50	0.42	0.57	0.70	0.64	0.62	0.59
Gene Diversity (He)		0.58	0.59	0.66	0.62	0.66	0.64	0.65	0.63

Locus LLSD4	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		34	8	12	22	10	11	43	
Number of alleles		10	6	6	7	8	7	7	7.29
	0	0.09	0.06	-	-	0.15	0.14	0.13	
	4	0.06	-	0.04	0.23	-	0.05	0.11	
	6	0.04	-	0.04	0.02	0.10	0.05	-	
	8	-	-	-	-	0.05	-	-	
	10	-	-	-	0.07	-	-	-	
	12	0.41	0.63	0.63	0.46	0.25	0.46	0.27	
	14	0.10	0.13	-	0.16	0.10	0.18	0.45	
	16	0.04	-	-	0.02	0.10	-	0.01	
	18	0.18	0.06	0.17	-	0.10	-	0.02	
	20	-	0.06	-	-	-	-	-	
	22	0.03	-	-	-	-	-	-	
	28	0.03	0.06	0.08	0.05	0.15	0.09	0.01	
	30	-	-	-	-	-	0.05	-	
	32	-	-	0.04	-	-	-	-	
	34	0.02	-	-	-	-	-	-	
Heterozygote Proportion (Ho)		0.77	0.63	0.50	0.77	0.90	0.91	0.72	0.74
Gene Diversity (He)		0.78	0.62	0.59	0.73	0.90	0.76	0.70	0.73

Table I. Continued.

Locus LLSD9	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		36	8	12	22	11	11	44	
Number of alleles		4	3	3	2	4	3	4	3.29
	0	0.03	0.19	0.04	-	0.05	0.05	-	
	2	-	-	-	-	-	-	0.09	
	8	0.79	0.75	0.83	0.84	0.64	0.86	0.73	
	10	0.04	-	-	-	0.05	-	0.09	
	12	0.14	0.06	0.13	0.16	0.27	0.09	0.09	
Heterozygote Proportion (*Ho*)		0.36	0.50	0.33	0.23	0.55	0.27	0.50	0.39
Gene Diversity (*He*)		0.36	0.43	0.30	0.27	0.54	0.26	0.45	0.37

Locus BG2	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		36	7	10	21	11	11	44	
Number of alleles		5	5	4	6	4	5	5	4.86
	0	-	-	-	0.02	-	-	-	
	4	-	-	-	0.19	0.14	0.14	0.16	
	8	0.01	0.07	-	-	-	-	-	
	12	0.08	0.36	0.20	0.19	0.27	0.27	0.14	
	16	0.17 0.21	0.25	0.29	0.46	0.23	0.24		
	20	0.64	0.29	0.50	0.21	0.14	0.32	0.39	
	24	0.10	-	0.05	0.10	-	0.05	0.08	
	32	-	0.07	-	-	-	-	-	
Heterozygote Proportion (*Ho*)		0.50	1.00	0.80	0.86	0.73	0.46	0.82	0.74
Gene Diversity (*He*)		0.56	0.79	0.68	0.81	0.71	0.79	0.75	0.73

Table I. Continued.

Locus BG5	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		37	8	12	22	11	11	43	
Number of alleles		10	5	6	8	6	5	6	6.57
	0	0.18	0.38	0.21	0.05	-	-	0.01	
	4	0.27	0.38	0.17	0.32	0.36	0.27	0.27	
	8	0.15	0.06	0.25	0.09	0.23	0.46	0.35	
	12	0.08	-	-	0.14	0.09	0.18	0.17	
	16	0.03	0.06	-	0.09	-	-	-	
	20	-	-	0.04	-	-	-	0.01	
	24	0.10	0.13	0.13	0.27	0.23	0.05	0.19	
	28	0.10	-	0.21	0.02	0.05	0.05	-	
	32	0.03	-	-	-	-	-	-	
	36	0.07	-	-	0.02	0.05	-	-	
	40	0.01	-	-	-	-	-	-	
Heterozygote Proportion (Ho)		0.68	0.88	0.83	0.82	0.73	0.91	0.67	0.79
Gene Diversity (He)		0.86	0.74	0.84	0.80	0.79	0.71	0.75	0.79

Locus TTT1	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		35	8	11	22	11	11	45	
Number of alleles		6	5	5	7	6	7	6	6.00
	238	-	-	-	0.02	-	-	-	
	242	-	-	0.09	0.05	0.14	0.23	0.16	
	246	0.39	0.25	0.18	0.34	0.14	0.05	0.18	
	250	0.06	0.06	-	0.16	0.18	0.18	0.14	
	254	0.39	0.56	0.55	0.25	0.27	0.23	0.32	
	258	0.07	0.06	0.09	0.16	0.23	0.18	0.12	
	262	0.07	0.06	-	0.02	0.05	0.09	0.08	
	266	0.03	-	0.09	-	-	0.05	-	
Heterozygote Proportion (Ho)		0.71	0.50	0.64	0.73	0.73	0.82	0.80	0.70
Gene Diversity (He)		0.70	0.65	0.68	0.79	0.84	0.86	0.81	0.76

Table I. Continued.

Locus TTT2	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		36	8	12	22	11	11	42	
Number of alleles		6	5	5	5	6	3	6	5.14
	179	-	-	-	-	0.18	-	0.01	
	183	0.10	-	0.13	0.02	0.05	-	0.01	
	187	0.08	0.06	0.08	0.14	0.32	0.23	0.49	
	191	0.14	0.31	0.04	0.25	0.14	0.18	0.06	
	195	0.47	0.38	0.58	0.43	0.23	0.59	0.29	
	199	0.19	0.19	0.17	0.16	0.09	-	0.14	
	203	0.01	0.06	-	-	-	-	-	
Heterozygote Proportion (Ho)		0.67	0.63	0.75	0.73	0.82	0.82	0.74	0.74
Gene Diversity (He)		0.71	0.77	0.63	0.72	0.82	0.59	0.66	0.70

Locus TTD2	Allele	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende	Means
Number of individuals		37	8	12	22	10	11	45	
Number of alleles		5	3	3	4	4	4	4	3.86
	150	0.01	-	-	-	-	-	-	
	152	0.30	0.50	0.33	0.14	0.15	0.18	0.08	
	154	0.19	0.06	0.17	0.27	0.15	0.18	0.27	
	156	0.46	0.44	0.50	0.36	0.40	0.09	0.22	
	160	0.04	-	-	0.23	0.30	0.55	0.43	
Heterozygote Proportion (Ho)		0.76	0.63	0.50	0.73	0.70	0.73	0.80	0.69
Gene Diversity (He)		0.67	0.59	0.64	0.74	0.74	0.66	0.69	0.68

All loci	Aravis/Beaufortain	Vanoise	Maurienne	Queyras	Haut-Var	Tinée	Tende
Mean Allele Number (allelic diversity)	6.38	4.50	4.50	5.38	5.25	4.75	5.25
Mean observed heterozygote proportion	0.64	0.66	0.60	0.68	0.73	0.69	0.71
Mean expected heterozygote proportion proportion	0.65	0.65	0.63	0.69	0.75	0.66	0.68
P	0.46	0.94	0.74	0.69	0.51	0.93	0.67

Table II. Multilocus estimates of Weir and Cockerham (1984) [28] F_{st} between population pairs. Values corresponding to significant levels of genotypic differentiation (Exact Tests) are given in bold type. The significant level (was set at 0.002 (Bonferroni correction for multiple testing). For comparison Slatkin R_{st} [25] are given in parentheses.

	Aravis/ Beaufortain	**Vanoise**	**Maurienne**	**Queyras**	**Haut-Var**	**Tinée**
Vanoise	0.019 (0.035)					
Maurienne	0.003 (–0.016)	0.010 (0.033)				
Queyras	**0.047** (0.046)	**0.038** (0.127)	**0.052** (0.051)			
Haut-Var	**0.057** (0.027)	0.042 (0.082)	**0.056** (0.013)	**0.047** (0.022)		
Tinée	**0.093** (0.044)	**0.092** (0.031)	**0.081** (0.021)	0.029 (0.020)	0.033 (0.009)	
Tende	**0.088** (0.055)	**0.102** (0.092)	**0.102** (0.062)	**0.039** (0.015)	**0.031** (0.019)	**0.020** (–0.012)

these landscape features apparently do not totally preclude gene flow. A substantial proportion of gene flow could result from movements occurring along forested drainages and/or across passes below 2500 m. Unsuitable habitat has been found to be highly effective in limiting dispersal of some other grouse. In red grouse, genetic data suggest that dispersal can be hindered by unsuitable habitats such as forests and agricultural lands less than 2 km wide [21]. Hazel grouse (*Bonasa bonasia*) are reportedly reluctant to cross areas of open habitat only several hundred meters wide, such as meadows or cultivated fields [1].

Because of their exceptional capacity of movement, birds usually exhibit less geographical difference in genetic structure than is found in fishes, reptiles or mammals [2]. Our results support this trend despite the fact that grouse move over shorter distances than do many other birds, for example passerines [6, 19, 22]. However, very high levels of genetic structure (up to 60% of differentiation between localities 50 km apart) have been reported in red grouse [21]. This is surprising both because dispersal propensities and life history traits of red grouse are similar to those of black grouse [14]. Although

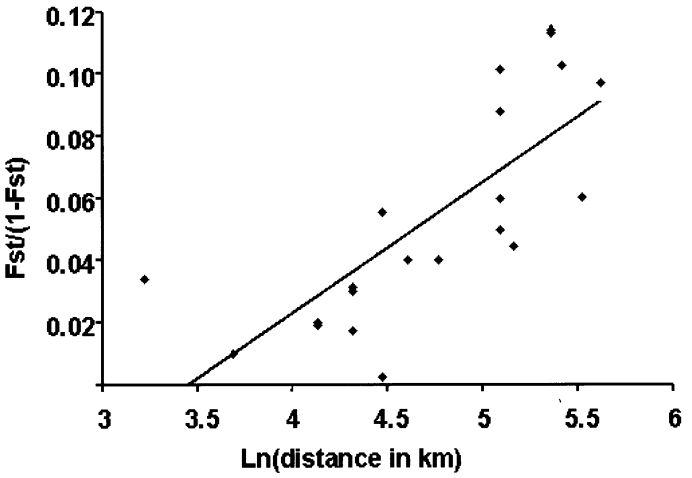

Figure 2. Relationship between the euclidean geographical distances between pairs of populations and the corresponding levels of genetic differentiation ($r^2 = 0.57$, regression line: $y = 0.042 \ln(x) - 0.11$).

Piertney *et al.* (1998) [21] interpreted these high levels of genetic differentiation as a consequence of high dispersal costs, one cannot exclude a bias due to the use of Slatkin R_{st} [26] instead of F_{st} as a measure of population differentiation. More particularly, R_{st} may have given unreliable estimates of genetic differentiation if the pattern of mutation did not follow the SMM model [26]. To be able to compare our results with those of Piertney *et al.* (1998) [21], we also estimated R_{st} values (Tab. II). Although R_{st} values varied within the same range of F_{st}, there was little consistency between the two estimates of population differentiation in black grouse.

Although relatively few individuals were genotyped, substantial variability was found at each locus (5 to 15 alleles per locus). Unfortunately, we cannot properly interpret these results because previous genetic studies on black grouse were performed with other markers (allozymes) [25] or with different sets of microsatellite loci [12]. Comparable levels of allelic diversity were found at loci LLSD4 and LLSD9 in healthy (dense and stable) populations of red grouse (LLSD4, 11 alleles in red grouse *versus* 15 in black grouse; LLSD9, 8 alleles in red grouse *versus* 5 in black grouse). However, this does not mean that black grouse populations in the French Alps are as viable as red grouse populations, because recent declines and isolation of black grouse may have reduced genetic variation. That is why we need to compare the genetic variation of black grouse in the Alps with that of populations in Fennoscandia or Russia. If the variation turned to be very low in the French Alps, one might envisage measures for restoring it.

Finally, there was a trend toward a decrease in allelic diversity in southernmost localities. This trend, which needs to be confirmed with larger samples, might reflect a "peninsula" or a "border" effect.

5. CONCLUSIONS

We present for the first time data on the genetic differentiation of populations of black grouse in the Alps. Although suitable habitats are discontinuous and potential barriers to dispersal are present, for example high rocky ridges, no evidence of any disruption in the distribution was found. Thus substantial gene flow occurs between populations, meaning that factors having local detrimental effects can have an influence on a much larger scale and therefore that future management actions must be envisaged over very large areas.

This study provides a basis for future monitoring of genetic variation in black grouse populations. Such monitoring will be particularly valuable if populations continue to decline.

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