

Genotyping faeces reveals facultative kin association on capercaillie's leks

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Abstract

The role that kin selection might play in the evolution of lekking in birds remains controversial. Recent molecular data suggest that males displaying on leks are related. Here we investigated the genetic structure and pattern of relatedness on leks of a declining population of capercaillie (*Tetrao urogallus*) using microsatellite genetic markers. Since the species is highly sensitive to disturbance, we adopted a non-invasive method by using faecal samples collected in the field. Based on a dataset of 50 males distributed in 6 sub-populations, we found significant genetic structuring among sub-populations, and a significant pattern of isolation by distance among leks. Estimates of relatedness showed that males displaying on the same lek were related, even when controlling for the effects of genetical differentiation among sub-populations. In addition, the frequency distribution of relatedness values indicated that leks contain a mixture of close kin and unrelated individuals (34 and 66%, respectively). This pattern is consistent with the hypothesis that leks often contain kin associations, which might be due to very restricted dispersal of some of the males or to joint dispersal of kin. The results are discussed with respect to their implication for the conservation of endangered populations.

Introduction

The evolution of lekking as a mating system remains a puzzling issue and a debated question in sexual selection theories (Andersson 1995; Høglund 2003). Leks are patches of territories on which males gather together during the mating season, and display with a ritualized courtship in order to attract females. Females mate preferentially with dominant males, resulting in a potential skew in males reproductive success (Fiske et al. 1998). Until recently, direct fitness benefits and competition among males have been considered to be the main factors driving the evolution of lek mating. However, because females usually prefer to mate in larger male aggregations rather than in small

leks, it has been proposed that low-rank males could enhance their inclusive fitness by increasing lek size of related high-rank males (Kokko and Lindström 1996). Indeed, the idea that kin selection could play an important role in the evolution and maintenance of lekking has received empirical supports from at least four bird species. Male peacocks (*Pavo cristatus*) were more closely related to other males within the same lek than to males in other leks (Petrie et al. 1999). Similarly, molecular data on lekking black grouse (*Tetrao tetrix*) (Høglund et al. 1999), lesser prairie-chicken (*Tympanuchus pallidicinctus*) (Bouzat and Johnson 2004) and white-bearded manakin (*Manacus manacus*) (Shorey et al. 2000) have revealed that relatedness was high among males sharing the

same lek. However, genetic similarity between males on leks may also result from limited male dispersal (philopatry) independently from kin selection (Hoglund 2003).

Lekking strategy and kin structures can have important consequences for conservation. Indeed, if the mating success is highly skewed towards a small number of males in the lek, effective population size (N_e) is expected to decrease, amplifying stochastic genetic processes such as drift. Moreover, kin association on leks might increase the level of inbreeding. The resulting loss of genetic diversity in small and fragmented populations of threatened species is then expected to reduce the ability to evolve and thus to increase extinction risks due to environmental changes (Frankham et al. 2002). In this context, Møller (2003) emphasized the importance of including sex when theoretically studying extinction risks in conservation biology.

Capercaillie (*Tetrao urogallus*), the largest European grouse, occurs in boreal or mountain forests. The species has highly specific habitat requirements, such as the presence of coniferous trees, open structures with moderate canopy cover and rich ground vegetation. In winter, capercaillies are arboreal and feed on coniferous needles, whereas in spring and summer they live on the ground and feed on leaves, seeds and fruits. Direct observations and telemetry suggest that most individuals, and particularly males, do not disperse far (Storch 1995; Storch and Segelbacher 2000). Populations of western and central Europe have shown a strong decline during the last decades, owing to habitat losses, habitat fragmentation and human disturbance (Storch 2000). The relict Jura population is distributed over ca. 550 km² in France and Switzerland, and is thought to contain approximately 500 breeding adults, with a balanced sex-ratio (Sachot 2002).

The aim of the present study was to investigate the genetic structure within and between leks of this declining population of capercaillie, as well as to investigate if males on leks cluster preferentially with kins. Because capercaillies are particularly sensitive to human disturbance and because the population of the Swiss and French Jura mountains has drastically declined since 20 years (Sachot et al. 2002), we used a non-invasive approach based on the genotyping of faeces.

Material and methods

Sampling

The study was conducted across the south-western portion of the Swiss and French Jura Mountains (Figure 1). We collected capercaillie faecal samples under deciduous and fir trees where individuals spend the night or feed, and also in 15 lek sites precisely identified by field observations during the breeding season (faeces from lekking birds were collected immediately after they had left the leks in the morning). The average distance among leks was 17 ± 12 km. The samples were distributed among nine discrete sub-populations defined as continuous patches of suitable habitat and determined by habitat suitability analysis (Sachot and Perrin 2004), of which six contained the lek sites (Figure 1 and Table 1). Faeces were individually dried in vials containing silica gel beads, and processed in a separated laboratory dedicated to low-content DNA samples.

Faecal samples DNA extraction and genotyping

We extracted DNA from 340 faecal samples. DNA extraction was performed with the QIAamp Stool kit (QIAGEN), using an optimized protocol (Regnaut et al. in press). We amplified 11 micro-satellite loci, 7 of which had been specifically designed for capercaillie (Segelbacher et al. 2000) and 4 for the black grouse *T. tetrix* (Caizergues et al. 2001). We monitored DNA contamination by adding negative controls in both the extraction and amplification experiments. Amplification products were visualized using an ABI377 automated sequencer (Applied Biosystems). In order to minimize genotyping errors due to the analysis of non-invasive samples with low or degraded DNA content (i.e. allelic dropout, false alleles), we adopted a multitube approach (Taberlet et al. 1996). Heterozygous individuals were genotyped twice and homozygous four times. Based on measures of allelic dropout and false alleles rates, using computer simulations with the software Gemini (Valière et al. 2002) we estimated the reliability of the genotyping procedure to be as high as 98% with five genotyping repetitions. Probabilities of identity at the 11 loci were low enough to assume that levels of chance matches were negligible, thus allowing for individual

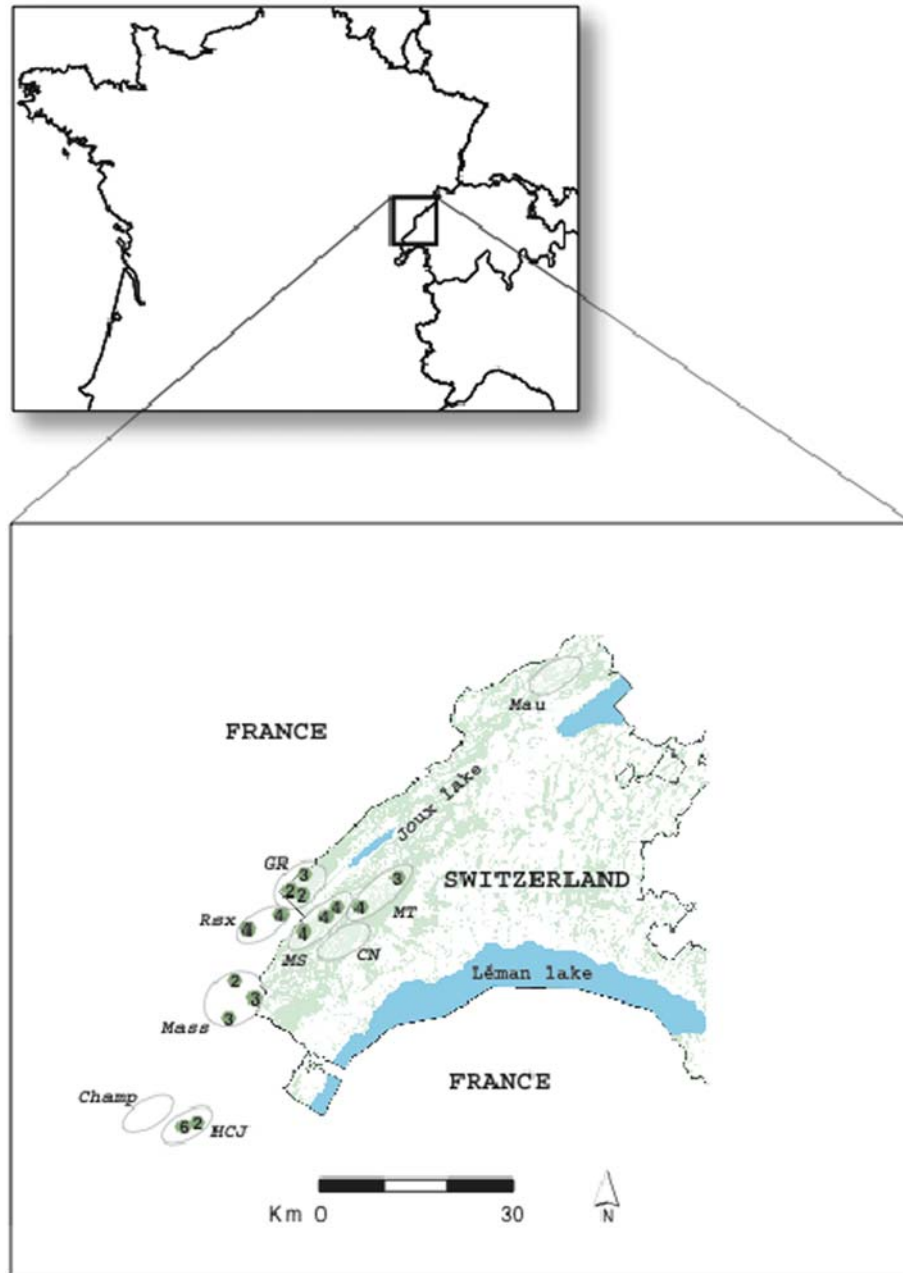


Figure 1. Sampling area and location of sub-populations and leks. Ovals correspond to the nine studied sub-populations (codes as in Table 1); small circles correspond to leks with number of lekking males.

identification (details in Regnaut et al. (in press)). In addition, genotypes differing by only one allele were considered as belonging to the same individual. Sex was determined by a PCR-based method adapted from Fridolfsson and Ellegren (1999). Two replicate PCRs were carried out for

each sample which was scored as male (one single band). Results of molecular sexing were also confirmed by morphological criteria (stool size; see Leclercq 1987). Detailed protocols of all molecular analyses and feasibility assessment are given in Regnaut et al. (in press).

Table 1. List of sub-populations and samples of *T. urogallus* analyzed in the present study

Sub-population	Code	<i>N</i>	Males	Males (lek)	Females	Unknown
Haute Chaîne du Jura	<i>HCJ</i>	38	29	8	8	1
Risoux	<i>Rsx</i>	24	11	8	10	3
Massacre	<i>Mass</i>	14	8	8	5	1
Mont Tendre	<i>MT</i>	45	31	7	10	4
Grand Risoux	<i>GR</i>	31	21	7	9	1
Mont Sala	<i>MS</i>	48	34	12	12	2
Crêt de la Neuve	<i>CN</i>	25	11	0	13	1
Champfromier	<i>Champ</i>	6	2	0	3	1
Mauborget	<i>Mau</i>	7	7	0	0	0
Total		238	154	50	70	14

N: nr. of samples; Males: nr. of males; Males (lek): nr. of males within lek; Females: nr. of females; Unknown: nr. of samples for which sex has not been identified.

Genetic data analysis

Gene diversities (heterozygosities), deviation from random mating within populations (*F_{is}*) per locus and sample and deviation from Hardy Weinberg Equilibrium (HWE) within samples were estimated and tested for significance with FSTAT 2.9.3.2 (Goudet 1995). Population genetic structure was investigated with a hierarchical analysis of variance, using the FSTAT software. Nei's distances *D* (1978) were computed with the GENETIX software (Belkhir et al. 1996–2004). The significance of the correlation between geographical distance and Nei's genetic distance was tested with a Mantel test using FSTAT.

We measured the relatedness (*r*) among males lekking together using the software RELATEDNESS 5.0 (Queller and Goodnight 1989), first with respect to the allele frequencies in the whole population as a reference (the 238 males and females; see below), and second with respect to the allele frequencies in each local sub-population (males and females). The 95% confidence intervals (CI) were obtained by jackknifing over loci.

To estimate pairwise relatedness among males in the same lek we used the software KINSHIP (Goodnight and Queller 1999). We also obtained the distribution of relatedness values for expected relationships by simulating 5000 pairs of unrelated or full-sib individuals, respectively, drawn from a population with the observed microsatellite allelic frequencies. In addition, we used a likelihood approach to determine which distribution of two relatedness classes best fitted the observed distribution.

Results

Population genetic structure

The number of alleles per locus ranged from 3 to 17 (average = 9), with a total of 101 alleles across 11 loci. Expected heterozygosities per locus within sub-populations (*H_s*) ranged from 0.28 to 0.745, with an average of 0.55. Randomization tests indicated that eight loci were at HWE, two loci had an heterozygous deficit and one locus had a heterozygous excess. We repeated all analyses without the three loci which deviated from HWE, which returned qualitatively similar results and did not affect the conclusions. In order to exploit all available information, we present here the results obtained with 11 loci. Expected overall heterozygosity (*H_t*) averaged 0.57 (range per locus: 0.29–0.78). Observed heterozygosity (*H_o*) ranged from 0.28 to 0.73, with an average of 0.535. There was evidence for significant deviation from random mating in none of the nine sub-populations (global *F_{is}* = 0.019). *F_{st}* among sub-populations was 0.033 over all loci, a value significantly greater than zero when randomising genotypes among sub-populations (*P* < 0.001).

Two hundred and thirty eight unique genotypes were scored throughout the whole sample set, of which 65% were genotyped as males, 29% as females and 6% as unknown (possibly due to DNA degradation; Table 1). The sex ratio was strongly male biased in the data set. We suggest that this is an effect of sampling, since collecting faeces on leks offers more opportunities to collect male samples, and since other counting methods did not

reveal unbalanced sex ratio in this population (Leclercq 2004). The male dataset consisted of 50 individuals in 15 leks within 6 sub-populations (see Figure 1; mean number of males \pm SE within leks = 3.3 ± 1.1), 84 males distributed outside leks in these 6 sub-populations, and 20 males within 3 sub-populations with no clear lek structure.

Isolation by distance

Isolation by distance was inferred from the dataset of 50 males in 15 lek sites. A pattern of isolation by distance was detected among leks, as shown by the significant correlation between Nei's genetic distance and geographic distance ($r^2 = 0.43$, $P < 0.001$, Mantel test with 20,000 randomizations; Figure 2).

Relatedness among males on leks

The relatedness among males sampled on the same lek was as high as 0.22 when measured with respect

to the entire population (the 238 males and females, Figure 3). This value decreased to 0.12 when the relatedness was measured with respect to the neighbouring sub-populations, thus controlling for the effect of genetic differentiation among sub-populations (Figure 3). Both values were significant, as shown by the 95% CI that do not overlap with zero.

Within sub-populations, the pairwise relatedness between males on the same lek ranged from -0.45 to 0.62 (Figure 4). Interestingly, the frequency distribution of relatedness values suggests a combination of two underlying unimodal gaussian curves, one centred on zero as expected for unrelated individuals, and one around 0.4, which lies between the theoretical values for half-sibs (0.25), and full-sibs or fathers-sons (0.5). This pattern is significantly different from the distribution obtained by simulating unrelated pairs of individuals with the observed microsatellite allele frequencies (Mann-Whitney $U_{76,5000} = 161,982$,

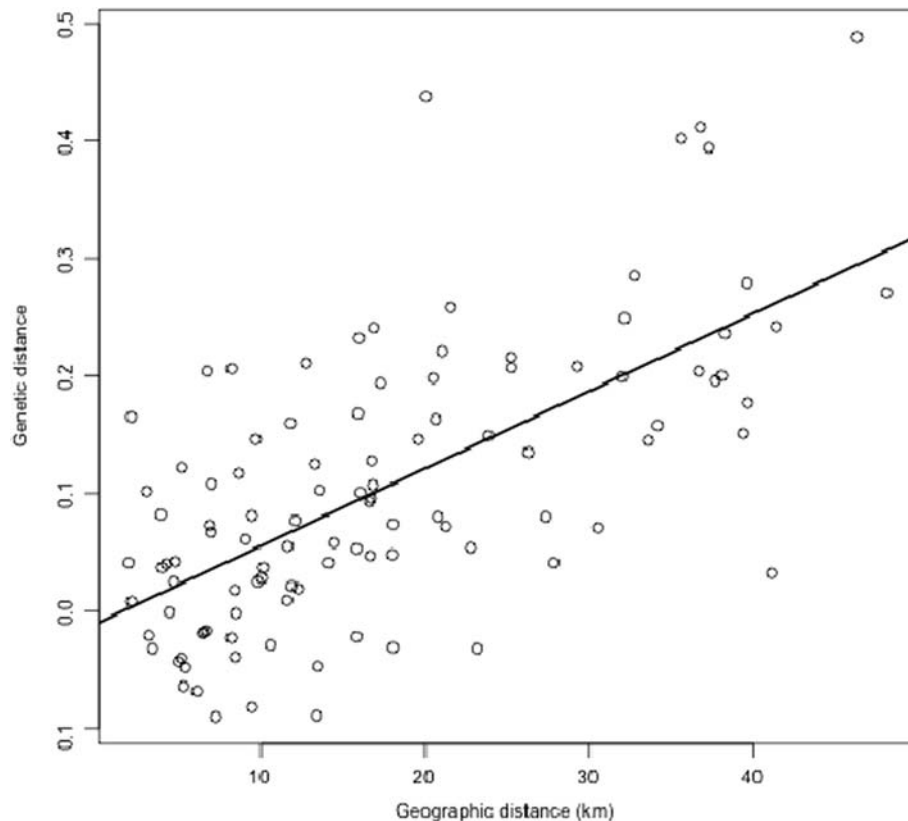


Figure 2. Correlation between Nei's genetic distances and geographic distances among leks for 50 males of *T. urogallus* distributed in 15 lek sites ($r^2 = 0.43$, $P < 0.001$).

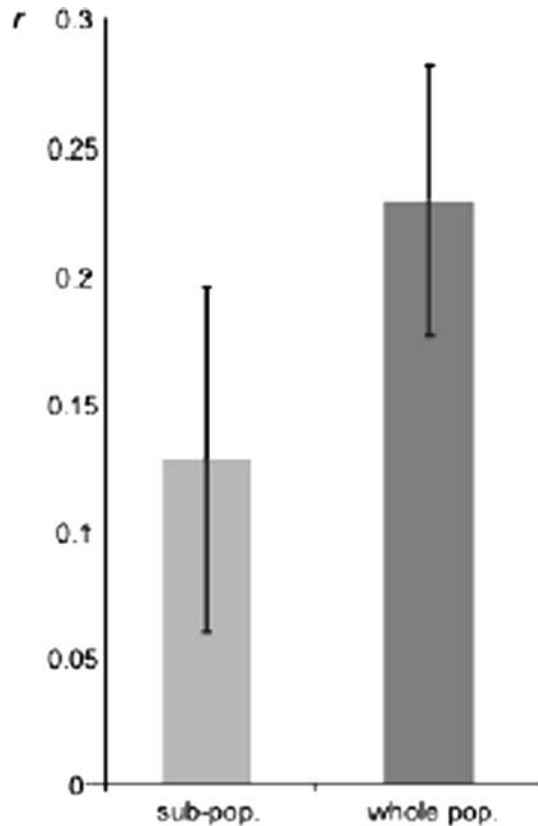


Figure 3. Average pairwise relatedness (r) among males from the same leks, measured with respect to allele frequencies in the local sub-populations (light grey bar), and with respect to allele frequencies in the whole population (dark grey bar). The 95% CI obtained by jackknifing over loci do not overlap with zero (vertical lines).

$P=0.027$; Figure 4). The observed frequency distribution of pairwise relatedness best fitted a distribution in which 66% of the pairs of males were unrelated and 34% were highly related ($r=0.4$; likelihood = -11.9).

Discussion

As in most of the species range in western Europe, the capercaillie (*T. urogallus*) population studied here has been drastically declining in the last decades, due to habitat loss and human disturbance (Sachot et al. 2002; Leclercq 2004). Hence, capturing free-ranging individuals for genetic sampling during reproductive period would have been dangerous for population survival. Here we used a non-invasive genetic approach to estimate

population parameters such as individual and sex identification, relatedness estimates and levels of genetic differentiation that are important for the conservation of this sensitive species (see Regnaut et al. in press).

We found that male capercaillie displaying on the same lek are significantly related and that leks contain a mixture of close kin and non-kin. These results have implications for the understanding of lekking and for the conservation of these highly endangered populations.

The simplest explanation for this genetic similarity on leks is that males' dispersal is restricted to short distances. Indeed, our results show a significant genetic differentiation among sub-populations and a strong pattern of isolation by distance among leks, which indicates that males have a higher probability of establishing on leks close to their natal sites. This is in agreement with field data indicating that male capercaillies do not disperse more than a few kilometres (Storch 1995). Isolation by distance has also been documented in other grouse populations (Piertney et al. 1998; Caizergues et al. 2003a, b) and in capercaillie at larger spatial scales (Segelbacher and Storch 2002; Segelbacher et al. 2003).

This study adds to a growing body of evidence that males on leks are often close relatives (Hoglund et al. 1999; Petrie et al. 1999; Shorey et al. 2000; Bouzat and Johnson 2004). In some species, the high relatedness of males might be due to kin association by phenotype matching (Petrie et al. 1999; Shorey et al. 2000). In some other species, genetic similarity might simply be explained by very limited dispersal of males and fidelity to lek sites (Hoglund et al. 1999; Bouzat and Johnson 2004). Whatever the mechanism, genetic structuring among leks seems common in lekking bird species, suggesting that there is a potential for kin selection to act in the maintenance of this mating system (Saether 2002; Hoglund 2003).

We found evidence that both close kin and unrelated male capercaillies share the same leks. Approximately 34% of the pairs of males on leks were highly related, often to the level of half-sibs, full-sibs or fathers-sons, when compared to random individuals from the same sub-populations. However, the rest of the pairs of cocks were unrelated. This pattern is consistent with the hypothesis that pairs of males often result from

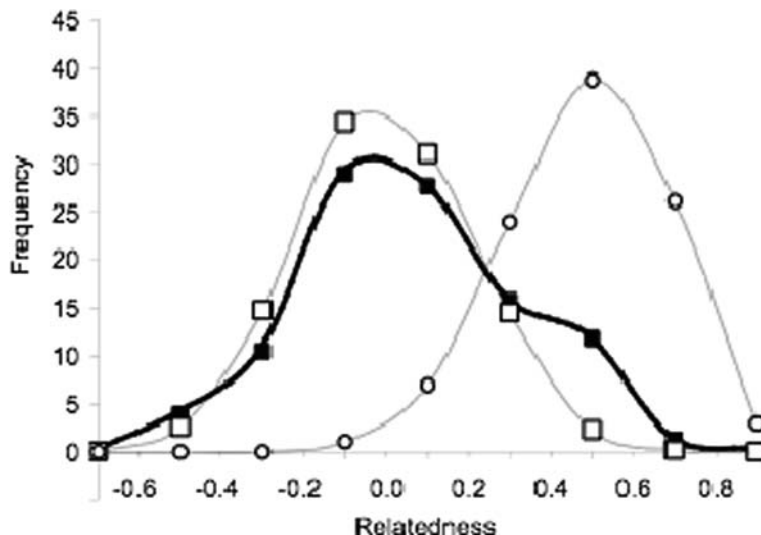


Figure 4. Observed relatedness distribution for pairs of males from the same leks, measured with respect to allele frequencies in the local sub-populations (filled squares), and simulated values for unrelated (open squares) and full-sib (open circles) individuals.

kin association, which might be due to very restricted dispersal of part of the males, or to joint dispersal of kin. However, indirect measures such as the distribution of genetic diversity within and among leks can only bring partial insight into the factors that concur to the establishment and maintenance of lekking behaviour. Hence more studies are needed to evaluate the direct and inclusive fitness benefits of males lekking together. In particular, it would be interesting to study if male capercaillie modify their social behaviour accordingly to their degree of relatedness to neighbours, as has been suggested in red grouse (Watson et al. 1994).

Information on parentage is crucial to study the impact of inbreeding in threatened species, to determine N_e , and to verify pedigrees used in genetic management. The mating system of the capercaillie, with leks, restricted dispersal and probably kin association of males, has a strong effect on the distribution of genetic diversity within populations. In association with the very small current population size and recent habitat fragmentation, the mating system might tend to increase drift and loss of genetic diversity within sub-populations. Our results show a significant level of genetic differentiation (global F_{st}) at this small spatial scale, suggesting genetic drift within semi-isolated sub-populations. The lek mating system together with the presence of

highly related individuals within leks might further lead to increased inbreeding and inbreeding depression. However, the presence of unrelated individuals might allow females to choose between mates with well-differentiated genotypes, and such choice might minimize inbreeding and its short-term deleterious outcomes. Thus, kin structure on leks should be taken into account in conservation plans such as the creation of artificial leks in captivity, translocation of individuals between populations, and reintroduction within sites with suitable habitat (Storch 2000).

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Appendix

Table A.1. Diversity indices for 11 microsatellite loci used in this study. Population codes as in Table

Locus	Population									
	HCJ	Rsx	MT	Mass	G	CN	MS	Champ	Mau	Means
TTTD6										
<i>A</i>	7	8	6	7	9	6	9	3	6	6.778
<i>R</i>	3.073	4.478	4.017	4.277	4.647	3.33	3.87	2.98	4.783	3.939
<i>Ho</i>	0.445	0.739	0.857	0.715	0.833	0.760	0.851	0.500	0.857	0.728
<i>Hs</i>	0.601	0.807	0.772	0.77	0.814	0.64	0.739	0.733	0.845	0.747
HWE										
TTD2										
<i>A</i>	9	8	3	7	11	5	7	2	4	6.222
<i>R</i>	4.532	3.122	2.362	3.847	5.024	3.681	3.757	1.998	3.392	3.524
<i>Ho</i>	0.656	0.522	0.364	0.618	0.700	0.695	0.721	0.667	0.713	0.629
<i>Hs</i>	0.81	0.531	0.577	0.734	0.838	0.724	0.72	0.467	0.69	0.677
HWE										
TTT1										
<i>A</i>	4	5	4	5	7	3	3	3	3	4.111
<i>R</i>	2.54	3.263	2.984	2.846	3.582	2.802	2.833	2.665	2.67	2.909
<i>Ho</i>	0.324	0.300	0.539	0.650	0.689	0.360	0.435	0.500	0	0.422
<i>Hs</i>	0.496	0.713	0.654	0.61	0.713	0.648	0.646	0.6	0.524	0.623
HWE										
TUT2										
<i>A</i>	2	3	2	2	4	2	2	2	2	2.333
<i>R</i>	1.995	2.299	1.986	1.981	2.251	1.994	1.994	1.998	1.985	2.054
<i>Ho</i>	0.500	0.250	0.286	0.533	0.666	0.375	0.490	0.667	0.286	0.450
<i>Hs</i>	0.507	0.549	0.484	0.475	0.518	0.505	0.505	0.467	0.452	0.496
HWE										
TUT3										
<i>A</i>	3	4	3	5	6	4	4	1	2	3.555
<i>R</i>	1.435	2.314	1.995	2.16	3.123	2.162	2.114	1	1.835	2.015
<i>Ho</i>	0.118	0.318	0.286	0.309	0.572	0.292	0.319	0	0.286	0.278
<i>Hs</i>	0.114	0.359	0.264	0.316	0.56	0.33	0.303	0	0.262	0.279
HWE										
TUD5										
<i>A</i>	6	7	4	6	5	4	8	3	3	5.111
<i>R</i>	3.405	3.762	2.727	3.143	3.233	3.321	3.178	3	2.516	3.143
<i>Ho</i>	0.731	0.800	0.500	0.635	0.792	0.750	0.745	1	0.571	0.725
<i>Hs</i>	0.678	0.716	0.481	0.592	0.656	0.673	0.612	0.625	0.464	0.611
HWE										
TTTD1										
<i>A</i>	6	5	3	6	7	5	5	3	1	4.555
<i>R</i>	3.015	2.775	1.995	3.481	3.297	3.129	2.991	2.818	1	2.722
<i>Ho</i>	0.464	0.435	0.286	0.763	0.607	0.524	0.681	0.666	0	0.492
<i>Hs</i>	0.503	0.492	0.264	0.692	0.621	0.582	0.61	0.533	0	0.477
HWE										
TUT4										
<i>A</i>	7	4	2	5	6	4	4	3	4	4.333
<i>R</i>	2.652	2.609	1.992	2.586	2.879	2.559	2.25	2.98	3.352	2.651
<i>Ho</i>	0.606	0.681	0.462	0.442	0.517	0.560	0.563	0.833	0.714	0.598

Table A.1. Continued

Locus	Population									
	HCJ	Rsx	MT	Mass	G	CN	MS	Champ	Mau	Means
<i>Hs</i>	0.529	0.554	0.494	0.486	0.546	0.491	0.457	0.7	0.643	0.544
HWE										
TUD1										
<i>A</i>	5	4	4	4	4	6	4	2	2	3.888
<i>R</i>	2.8	3.027	2.913	2.511	3.012	3.152	2.297	2	1.985	2.633
<i>Ho</i>	0.371	0.761	0.616	0.527	0.417	0.599	0.553	0.834	0.286	0.551
<i>Hs</i>	0.591	0.627	0.574	0.521	0.625	0.653	0.507	0.5	0.452	0.561
HWE								*		
TTTT1										
<i>A</i>	3	2	2	3	3	3	3	3	2	2.666
<i>R</i>	2.499	1.705	1.952	1.536	1.903	2.077	2.28	2.98	1.571	2.056
<i>Ho</i>	0.393	0.261	0.384	0.114	0.276	0.280	0.605	0.333	0.143	0.310
<i>Hs</i>	0.478	0.231	0.41	0.15	0.296	0.341	0.487	0.75	0.143	0.365
HWE		*							*	
TUT3										
<i>A</i>	3	4	3	4	4	4	4	4	3	3.666
<i>R</i>	2.722	3.21	2.739	2.834	2.883	3.12	2.755	3.331	2.82	2.935
<i>Ho</i>	0.555	0.546	0.643	0.5	0.807	0.880	0.724	0.834	0.857	0.705
<i>Hs</i>	0.595	0.668	0.61	0.624	0.64	0.676	0.601	0.667	0.595	0.631
HWE									*	
All loci										
Mean <i>A</i>	5	4.909	3.273	4.909	6	4.182	4.818	2.636	2.909	
Mean <i>R</i>	2.788	2.960	2.515	2.837	3.258	2.848	2.756	2.523	2.537	
Mean <i>Ho</i>	0.469	0.510	0.475	0.528	0.625	0.552	0.608	0.621	0.428	
Mean <i>Hs</i>	0.537	0.568	0.508	0.543	0.621	0.569	0.562	0.549	0.461	
HWE										

A: allele number; *R*: allelic richness; *Ho*: observed heterozygosity; *Hs*: expected heterozygosity; *HWE: deviates significantly from Hardy-Weinberg equilibrium after Bonferroni adjustment ($p < 0.05$).

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