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Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps

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Abstract

The genetic structure of the Alpine marmot, *Marmota marmota*, was studied by an analysis of five polymorphic microsatellite loci. Eight locations were sampled in the French Alps, one from Les Ecrins valley (n = 160), another from La Sassière valley (n = 289) and the six others from the Maurienne valley (n = 139). Information on social group structure was available for both Les Ecrins and La Sassière but not for the other samples. The high levels of genetic diversity observed are at odds with the results obtained using microsatellites, minisatellites and allozymes on Alpine marmots from Germany, Austria and Switzerland. Strong deficits in heterozygotes were found in Les Ecrins and La Sassière. They are caused by a Wahlund effect due to the family structure (i.e. differentiation between the family groups). The family groups exhibit excess of heterozygotes rather than deficits. This may be caused by outbreeding and this is compatible with recent results from the genetics of related social species when information on the social structure is taken into account. The observed outbreeding could be the result of females mating with transient males or males coming from neighbouring colonies. Both indicate that the species may not be as monogamous as is usually believed. The results are also compatible with a male-biased dispersal but do not allow us to exclude some female migration. We also found a significant correlation between geographical and genetic distance indicating that isolation by distance could be an issue in marmots. This study is the first that analysed populations of marmots taking into account the social structure within populations and assessing inbreeding at different levels (region, valley, population, and family groups). Our study clearly demonstrated that the sampling strategy and behavioural information can have dramatic effects on both the results and interpretation of the genetic data.

Keywords: *F*-statistics, inbreeding avoidance, isolation by distance, *Marmota marmota*, microsatellites, population genetic structure

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Introduction

The distribution of neutral genetic variability within populations or social groups and between them is the result of a balance between mutation, migration and random drift (Epperson & Li 1997). The mating system will also contribute in shaping this diversity within the individuals. In particular, behavioural processes are expected to play a key role in social species, in promoting or minimizing inbreeding (Greenwood 1980). However, the exact manner in which genetic variability can be explained in terms of behaviour can be quite complex and is still under considerable debate. For example, genetic analyses of black-tailed prairie dog populations and social groups has led different authors to classify the mating system as strongly inbred (Chesser 1983), slightly outbred (Foltz & Hoogland 1983) or neither (Daley 1992). A recent study suggests that the level of inbreeding or outbreeding in this species is strongly influenced by the basic unit analysed (family group vs. 'coterie' (social breeding groups in prairie dogs) vs.

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population, Dobson *et al*. 1997). This particular example suggests that a careful sampling strategy and good understanding of the units sampled is required when social species are studied using genetic analysis.

The Alpine marmot (*Marmota marmota*) is a highly social rodent that inhabits European Alps and lives in family groups composed of a dominant pair and their subordinates. Migration between groups has been observed (Arnold 1990a,b; Frey-Roos 1999; Magnolon 1999) but long-range migration is considered unlikely. Arnold (1990a,b) also observed frequent replacement of a dominant individual by an offspring. Thus, the small size of family groups and the limited amount of migration between them are expected to result in differentiation by genetic drift and perhaps inbreeding. The mating system is usually defined as monogamous (Zelenka 1965; Barash 1976; Arnold 1990a; Perrin *et al*. 1993). Only one male and one female are responsible for the birth of all individuals of one group. However, a recent genetic study has detected more than 30% of extra-pair copulation in a French population showing that monogamy is not exclusive in this species (Goossens *et al*. 1998a), and that outbreeding and gene flow may be more common than suspected.

Previous studies of Alpine marmot populations have used allozymes (Arnold 1990a; Preleuthner & Pinsker 1993) and DNA fingerprinting methods (Arnold 1990a; Rassmann *et al*. 1994) to evaluate genetic diversity, population structure and mating system. Arnold (1990a) found only two di-allelic allozyme systems among 53 allozyme loci tested for 386 individuals in a paternity analysis of one German population. Preleuthner & Pinsker (1993) also detected only two polymorphic loci out of 50 allozyme loci and estimated an average heterozygosity of 0.012 for populations from Austria and Switzerland. This result was confirmed by Preleuthner *et al*. (1995). These authors explained the low level of genetic diversity by population size reductions during the Pleistocene and local extinctions caused by overhunting. Rassmann *et al*. (1994) used multilocus DNA fingerprints (minisatellites) to study the German population of Alpine marmots analysed by Arnold (1990a). These authors found a limited degree of genetic variability (average $H_E = 0.12$) even though minisatellites are generally hypervariable (Burke *et al*. 1991). Rassmann *et al*. (1994) suggest that this was more likely the result of multiple recent bottlenecks rather than ancient Pleistocene bottlenecks. In particular, the high mortality rate (up to 18% in the 1989/1990 with an average of 8%) in the study site during harsh winters can cause the extinction of whole family groups (Rassmann *et al*. 1994).

In contrast, allozyme analyses in two other marmot species, *M. flaviventris* (Schwartz & Armitage 1980) and *M. monax* (Wright *et al*. 1987), have shown average heterozygosities of 0.075 and 0.053, respectively. Other closely related species such as ground squirrels (*Spermophilus beldingi*, Hanken & Sherman 1981) and prairie dogs (*Cynomys ludovicianus*, Chesser 1983) showed high allozyme variation with H_E around 0.107 and 0.066, respectively. Thus, allozyme diversity is much higher in all these species than in *M. marmota*.

The low genetic variability in Alpine marmot populations obtained with allozymes and minisatellites argues for the use of a more variable type of genetic marker. Microsatellite loci showed a substantial level of variation, even when studying isolated populations in wolf (*Canis lupus*, Ellegren *et al*. 1996) and in wallaby (*Petrogale assimilis*, Spencer *et al*. 1997), bottlenecked species such as koala (*Phascolarctos cinereus*, Houlden *et al*. 1996) and wombat (*Lasiorhinus krefftii*, Taylor *et al*. 1994), and endangered species such as the Ethiopian wolf (*Canis simensis*, Gottelli *et al*. 1994). The first study involving microsatellite loci on *M. marmota* was that of Kruckenhauser *et al*. (1997) on Austrian and Swiss populations. They used a microsatellite DNA probe to define multilocus profiles (i.e. multilocus DNA fingerprinting instead of single locus genotypes) and found a limited amount of genetic variation within these populations.

To summarize, analysis of *M. marmota* populations using allozymes, mini- or microsatellites indicates a limited level of genetic variation. Bottlenecks have been invoked as potential causes but it is not clear whether these bottlenecks are caused by recent (harsh winters, overhunting) or ancient (Pleistocene) demographic events.

In the present paper, we investigated the population structure of eight Alpine marmot populations from the French Alps that show varying degrees of geographical separation (Fig. 1) by typing six microsatellites. The objectives of our study were to: (i) assess the level of genetic diversity within populations; (ii) determine the extent of inbreeding or outbreeding within marmot populations; (iii) partition genetic diversity within these populations by taking into account information on family groups whenever it was available; and (iv) determine if genetic distance statistics for populations of marmots in France were consistent with an isolation by distance model.

Materials and methods

Sampling

The marmots were sampled from eight locations in the French Alps: one sample came from the National Park of Les Ecrins (Nature Reserve of Prapic $n = 160$), six came from the Maurienne valley (Aussois $n = 18$, Bessans $n = 23$, Bonneval $n = 30$, Chavière $n = 18$, Lans-le-Bourg $n = 26$, and Lans-le-Villard $n = 24$) and the last one came from the National Park of La Vanoise (Nature Reserve of La Sassière $n = 289$) (Fig. 1). All samples except those from Les Ecrins belong from the upland area called the *Massif de la Vanoise*. Individuals from Les Ecrins and La Sassière were trapped, marked and observed for behavioural

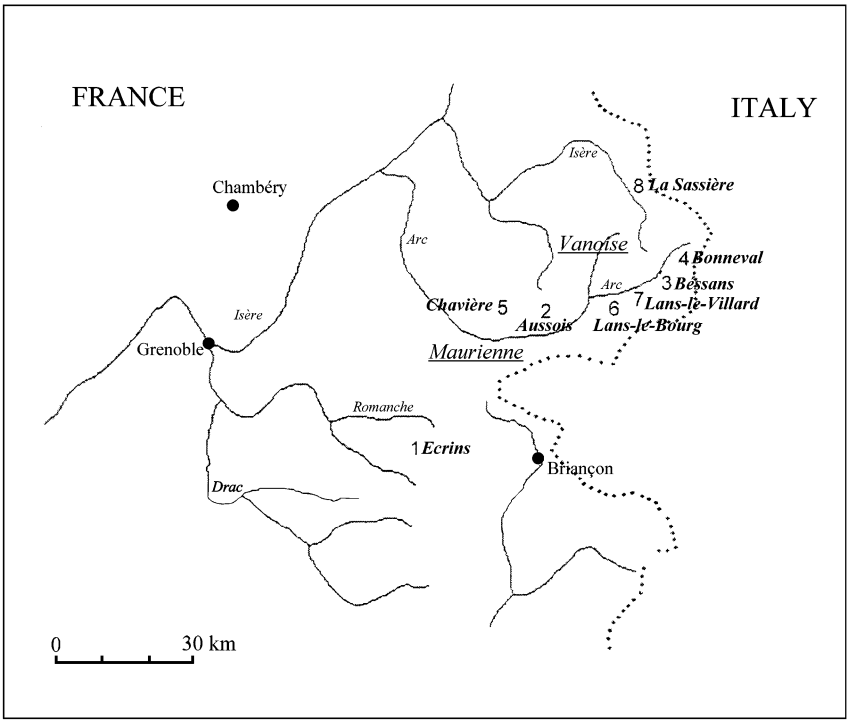

Fig. 1 Location of the eight samples in the French Alps. Populations: (1) Les Ecrins (2) Aussois (3) Bessans (4) Bonneval (5) Chavière (6) Lans-le-Bourg (7) Lans-le-Villard, and (8) La Sassière.

studies and genetic parentage analyses (Goossens *et al*. 1996, 1998a). Information on the community structure (family groups) was available for these populations (see Goossens *et al*. 1998a; for details on group sampling). Individuals from the other six populations ($n = 139$) were trapped in 1996 and information on family groups was either not available or unreliable and, therefore, was not used. Hairs (50–100) were plucked from each individual and scored in paper envelopes at room temperature until DNA extraction.

DNA extraction

DNA was extracted from 10 hairs per individual according to Goossens *et al*. (1998b) by cutting a 1-cm portion from the root end of the hair and placing it in 400 µL of a 5% Chelex-100 (Bio-Rad) suspension (Walsh *et al*. 1991). The samples were incubated at 56 °C for 6 h, and then in a boiling water bath for 8 min. Five µL of each extract were added as template in each polymerase chain reaction (PCR).

DNA amplification and microsatellite analyses

Six microsatellite DNA loci cloned and characterized in *Marmota marmota* (SS-Bibl1, SS-Bibl4, SS-Bibl18, SS-Bibl20, SS-Bibl25 and SS-Bibl31, Klinkicht 1993) were amplified with Amplitaq® Gold in a Perkin Elmer Gene Amp PCR System 9600 (primer sequences are given in Goossens *et al*. 1996). One primer from each pair was synthesized with a fluorescent dye group, FAM, HEX or TET, on the 5′ end. Combinations of three microsatellite loci giving sufficiently different size ranges were multiloaded (SS-Bibl1 + SS-Bibl25 + SS-Bibl31, and SS-Bibl4 + SS-Bibl18 + SS-Bibl20). Amplification of the SS-Bibl loci for each individual was carried out in 25 µL reaction (10 mM Tris-HCl (pH 9.0), 200 mM $(NH_4)_2SO_4$, 50 µM each dNTP, 1.5 mM $MgCl_2$, 5 ng BSA, 0.1 U Amplitaq® Gold DNA polymerase (Perkin Elmer), 0.5 µM (for FAM) or 0.75 µM (for TET) or 1 µM (for HEX) fluorescent primer, same concentration for the nonfluorescent primer). A PCR amplification of 50 cycles was carried out with initial denaturation at 95 °C for 10 min (95 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s). All positive PCR products were separated in a 6% Long Ranger™ gel (FMC) at 2670 V for 2h30 using an ABI PRISM™ 377 DNA sequencer (Perkin Elmer) with marker GS350 Tamra. All gels were analysed using GENESCAN™ Analysis 2.0, and GENOTYPER™ 1.1 software.

Statistical analyses

Genetic diversity was measured as the mean number of alleles per locus, observed (H_O), and expected (H_E) heterozygosities (Nei 1978). Wright's *F*-statistics were used to analyse within and between population structure. F_{IT} measures the departure from Hardy–Weinberg (HW) proportions at the level of the whole sample, while F_{IS} measures the part caused by departure at the level of individual samples and F_{ST} the part which is caused by differentiation between samples (Wright 1965).

Wright's *F*-statistics were estimated according Weir & Cockerham (1984) and their departure from the null hypothesis (no population differentiation for F_{ST}, and HW equilibrium for F_{IS} and F_{IT}) was tested using permutations as implemented in the GENETIX software program (Belkhir *et al*. 1996–1997; available at http://www.univ-montp2.fr/~genetix/genetix.htm). Linkage disequilibrium was measured using the average correlation coefficient as defined in Garnier-Géré & Dillmann (1992) based on the correlation coefficients between all pairs of alleles and tested using permutations in GENETIX.

Isolation by distance and correlation between genetic and geographical distance

In a model of isolation by distance (IBD), genetic distance between populations is expected to increase with geographical distance. Slatkin (1993) showed that, assuming that the mutation rate is much smaller than the migration rate, there is a decreasing linear relationship between $\text{Log}((1 - F_{ST})/4F_{ST})$ and Log(Geographic distance) which can be used to detect IBD visually and to estimate the amount of gene flow between populations.

One way to test the correlation between these two measures of distance is to use Mantel test (Mantel 1967). It is possible to measure this correlation by either using the original *Z*-statistic defined by Mantel or a normalization of *Z*, called *r* (Smouse *et al*. 1986), which is equivalent to an autocorrelation coefficient. We used *r* because its values are easier to interpret (it varies between –1 and 1). Note, however, that both gave the same results in terms of significance.

The Mantel test analyses were performed using two different measures of geographical distance. In the first case, geographical distances were estimated as straight-line distances between any pair of locations. The underlying assumption is that marmots disperse uniformly in any direction, whether or not there is a mountain between the locations. In the second case, geographical distance was estimated following the valleys. The underlying assumption being that marmots move preferentially along the valleys (for instance the distance between any sample in the Maurienne valley and La Sassière requires going first 'downstream' the Maurienne valley up to the point where the two valleys meet and then going 'upstream' up to La Sassière, Fig. 1). The Mantel test was performed using the Mantel option in the GENETIX software.

Results

Genetic variation and Hardy–Weinberg equilibrium

All six microsatellite loci were polymorphic, with the number of alleles per locus ranging from five (Locus SS-Bibl31) to 12 (Loci SS-Bibl18 and SS-Bibl20). The average number of alleles across populations varied between 4.2 and 6.3 (Table 1). Expected heterozygosities ranged from a minimum of 0.29 for SS-Bibl25 in Les Ecrins to a maximum of 0.81 for SS-Bibl18 and SS-Bibl20 in Bessans and Lans-le-Villard, respectively (Table 1). The average expected heterozygosity for all six loci ranged from 0.57 to 0.76 (i.e. much higher than that observed by Rassmann *et al*. 1994 in minisatellites, and Kruckenhauser *et al*. 1997 in multilocus microsatellite profiles). Departures from HW proportions as measured by F_{IS} values were significant in all populations (except Aussois and Bessans) when all loci were taken into account. However, this was mostly caused by locus SS-Bibl25 which exhibited strong and very significant heterozygote deficits in all samples except Aussois ($0.29 < F_{IS} < 0.91$, $P < 0.001$ in all cases, Table 1). These strong deviations from HW proportions can be attributed to the presence of null alleles, selection, or assortative mating (homogamy). Note that during the whole genotyping process and the paternity analysis (Goossens *et al*. 1998a) this locus did not exhibit any peculiar behaviour such as amplification or other technical problems and, therefore, null alleles are unlikely to be the major cause. However, because it is unclear which of these three hypotheses is the most likely it seemed more reasonable to exclude this locus from the rest of the analyses. When this locus was removed the average F_{IS} value dropped but was still significant ($F_{IS} = 0.05$, $P < 0.001$). Three of the four remaining significant F_{IS} values (Table 1) were observed in Les Ecrins and La Sassière, perhaps indicating a sample effect (see Discussion).

Linkage disequilibrium

When all populations were analysed together significant linkage disequilibrium (LD) was observed between all pairs of loci (26 significant values out of 80, Table 2). LD was particularly important within Les Ecrins and La Sassière samples (20 significant values out of 20, Table 2) but was also significant among six of the 60 remaining values. There is not any particular trend though, the six values being evenly distributed among the 10 pairs of loci (0, 1 or 2 values per pair). LD could be caused either by 'real' association (i.e. physical linkage or selection on certain multilocus genotypes) between alleles across loci or could be an artefact due to substructure (i.e. the presence of subgroups within some samples) (Ohta 1982). It is likely that all the samples of marmots obtained in this study were a mixture of family groups. However, it is only for Les Ecrins and La Sassière that this information was available and therefore could be tested by measuring the amount of differentiation between family groups (see below). Table 2 shows that the absolute values of correlation are usually larger in the samples from the Maurienne valley (range: 0.14–0.42, average: 0.22) than from Les

Table 1 Average number of alleles across populations (na), observed (H_O) and expected (H_E) heterozygosities and departures from Hardy–Weinberg proportions (F_{IS}) for all populations and for all loci. NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. n = sample size. Total-25 = mean values of H_O, H_E, and F_{IS} for all populations and all loci except SS-Bibl25

		SS-Bibl1	SS-Bibl4	SS-Bibl18	SS-Bibl20	SS-Bibl31	SS-Bibl25	na	Total	Total-25
Ecrins	H_E	0.55	0.71	0.61	0.80	0.43	0.29	6.33	0.57	0.62
n = 160	H_O	0.39	0.61	0.66	0.74	0.38	0.21		0.50	0.56
	F_{IS}	0.30***	0.15**	−0.08 NS	0.07 NS	0.11 NS	0.29***		0.12***	0.11***
Aussois	H_E	0.65	0.60	0.56	0.65	0.69	0.50	4.17	0.61	0.63
n = 18	H_O	0.67	0.61	0.61	0.72	0.89	0.33		0.64	0.70
	F_{IS}	−0.02 NS	−0.01 NS	−0.10 NS	−0.12 NS	−0.30 NS	0.34 NS		−0.05 NS	−0.11 NS
Bessans	H_E	0.80	0.67	0.81	0.80	0.72	0.78	6.00	0.76	0.76
n = 23	H_O	0.83	0.74	0.83	0.91	0.65	0.35		0.72	0.79
	F_{IS}	−0.04 NS	−0.10 NS	−0.02 NS	−0.15 NS	0.10 NS	0.56***		0.06 NS	−0.04 NS
Bonneval	H_E	0.72	0.47	0.71	0.79	0.67	0.49	4.83	0.64	0.67
n = 30	H_O	0.83	0.27	0.83	0.77	0.43	0.20		0.56	0.63
	F_{IS}	−0.17 NS	0.43***	−0.18 NS	0.02 NS	0.35 **	0.59***		0.13**	0.06 NS
Chavière	H_E	0.67	0.53	0.59	0.71	0.61	0.61	4.50	0.62	0.62
n = 18	H_O	0.56	0.61	0.67	0.72	0.61	0.06		0.54	0.63
	F_{IS}	0.17 NS	−0.15 NS	−0.14 NS	−0.02 NS	0.01 NS	0.91***		0.14*	−0.02 NS
Lans-le-Bourg	H_E	0.79	0.74	0.73	0.80	0.67	0.63	5.67	0.73	0.75
n = 26	H_O	0.92	0.65	0.65	0.89	0.69	0.15		0.66	0.76
	F_{IS}	−0.17 NS	0.12 NS	0.10 NS	−0.10 NS	−0.04 NS	0.76***		0.09*	−0.02 NS
Lans-le-Villard	H_E	0.78	0.69	0.75	0.81	0.66	0.39	5.83	0.68	0.74
n = 24	H_O	0.79	0.54	0.79	0.67	0.63	0.17		0.60	0.68
	F_{IS}	−0.01 NS	0.22 NS	−0.05 NS	0.18 NS	0.06 NS	0.58***		0.12**	0.08 NS
Sassière	H_E	0.67	0.52	0.72	0.73	0.68	0.63	5.50	0.66	0.66
n = 289	H_O	0.64	0.51	0.67	0.72	0.61	0.42		0.60	0.63
	F_{IS}	0.04 NS	0.02 NS	0.06 NS	0.004 NS	0.10**	0.33***		0.09***	0.05**
Total	H_E	0.79	0.61	0.78	0.83	0.66	0.76		0.74	0.73
n = 588	H_O	0.61	0.55	0.69	0.74	0.55	0.31		0.58	0.63
	F_{IS}	0.07	0.08	−0.001	0.02	0.09	0.40		0.10	0.05***

Table 2 Analysis of linkage disequilibrium (without SS-Bibl25). NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

	Les Ecrins	Aussois	Bessans	Bonneval	Chavière	Lans-le-Bourg	Lans-le-Villard	La Sassière	Total
SS-Bibl1/SS-Bibl4	0.089	0.297	0.245	0.235	0.217	0.218	0.317	0.081	0.070
	*	NS	*	NS	NS	NS	NS	*	***
SS-Bibl1/SS-Bibl18	0.078	0.281	0.205	0.202	0.196	0.198	0.168	0.070	0.094
	*	NS	NS	NS	NS	NS	NS	***	*
SS-Bibl1/SS-Bibl20	0.091	0.316	0.213	0.150	0.213	0.191	0.172	0.084	0.095
	***	NS	NS	NS	NS	NS	NS	***	NS
SS-Bibl1/SS-Bibl31	0.140	0.235	0.214	0.196	0.211	0.216	0.226	0.126	0.115
	**	NS	NS	NS	NS	NS	NS	***	**
SS-Bibl4/SS-Bibl18	0.216	0.200	0.215	0.147	0.257	0.164	0.206	0.112	0.048
	***	NS	NS	NS	NS	NS	NS	***	*
SS-Bibl4/SS-Bibl20	0.084	0.268	0.201	0.144	0.419	0.207	0.170	0.090	0.054
	*	NS	NS	NS	*	NS	NS	***	*
SS-Bibl4/SS-Bibl31	0.143	0.339	0.198	0.208	0.287	0.272	0.153	0.085	0.063
	***	*	NS	NS	NS	**	NS	***	*
SS-Bibl18/SS-Bibl20	0.076	0.319	0.265	0.142	0.218	0.200	0.172	0.093	0.074
	*	*	NS	NS	NS	NS	NS	***	*
SS-Bibl18/SS-Bibl31	0.157	0.228	0.248	0.182	0.309	0.209	0.195	0.123	0.089
	***	NS	*	NS	NS	NS	NS	***	***
SS-Bibl20/SS-Bibl31	0.101	0.196	0.150	0.197	0.210	0.192	0.204	0.115	0.073
	*	NS	NS	NS	NS	NS	NS	***	NS

Table 3 Pairwise F_{ST} (without SS-Bibl25). NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

	Ecrins	Aussois	Bessans	Bonneval	Chavière	Lans-le-Bourg	Lans-le-Villard	Sassière
Ecrins		0.252	0.160	0.199	0.261	0.177	0.165	0.203
Aussois	***		0.056	0.071	0.042	0.056	0.057	0.087
Bessans	***	***		0.019	0.046	0.023	−0.001	0.065
Bonneval	***	***	*		0.018	0.036	0.015	0.028
Chavière	***	**	**	NS		0.022	0.033	0.037
Lans-le-Bourg	***	***	**	**	*		0.009	0.062
Lans-le-Villard	***	***	NS	NS	*	NS		0.056
Sassière	***	***	***	***	***	***	***	

Ecrins and La Sassière (range: 0.07–0.22, average for Ecrins: 0.12, average for La Sassière: 0.10). Thus, it is possible that a number of these LD values are not significant because of a lack of power. This can be tested by analysing LD within the 32 family groups of Les Ecrins and La Sassière that are of similar size as the samples from the Maurienne valley. We found that these LD values are also larger (range 0.00–0.96, average for Ecrins: 0.37, average for La Sassière: 0.32, see Appendices I and II) indicating thus an effect of the sample size on the absolute value of LD. This effect is difficult to explain but seems to be a property of the estimator. Because we use permutations (which keep the sample size constant) to test for departures from zero, this effect is not a problem and significance levels can be used. We found that 28 out of the 320 values were significant. We did not find any trend such as significant LD for certain pairs of loci. The only effect seems that 15 of these 28 values are observed between three family groups (F, G and J, all from La Sassière, see Appendix II). We show below that substructure is a likely cause for the observed LD and this result is indirectly confirmed by the fact that these three family groups happen to be the family groups where the dominant pair changed every year, whether it was only the male, the female or both. As a consequence LD is most likely caused by substructure.

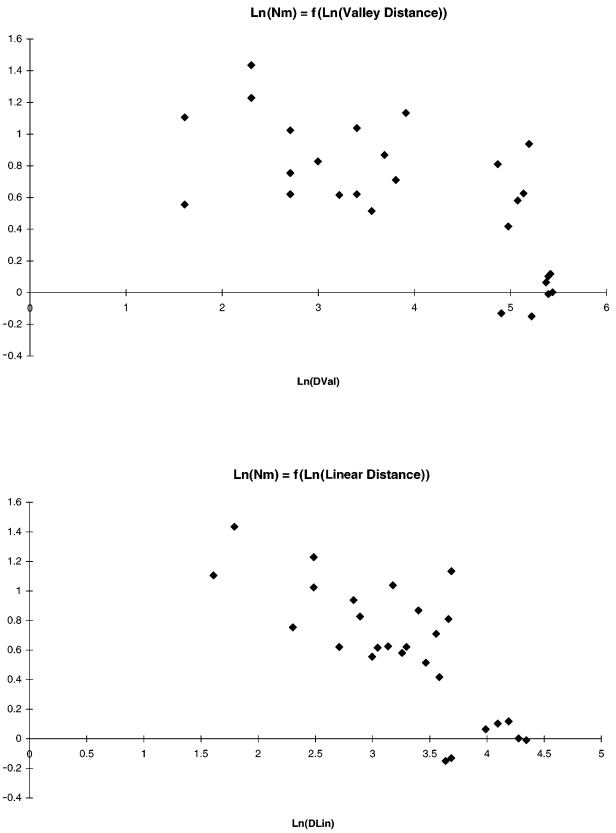

Fig. 2 Isolation by distance. Logarithm of Nm is plotted against the logarithm of the geographical distance. (a) The valley distance was used. (b) The linear distance was used.

Population structure and isolation by distance

Even though the F_{IS} values observed within populations were high and significant, most of the global departure from HW proportions ($F_{IT} = 0.186$) was caused by inter-population differentiation with a high and significant average $F_{ST} = 0.143$ ($P < 0.001$). Most of this differentiation is actually caused by Les Ecrins as can be seen from the pairwise F_{ST} values displayed in Table 3. This population exhibits F_{ST} values ranging between 0.16 and 0.26 with the other samples, while the next highest pairwise F_{ST} value not involving Les Ecrins is ≈0.09 (Table 3). This is not surprising given that Les Ecrins is the only sampled site outside the *Massif de la Vanoise*. The other pairwise F_{ST} values are not small given the geographical scale (Fig. 1), with the F_{ST} values between 0.02 and 0.09. La Sassière, the only sample outside the Maurienne valley, appears then as the most differentiated population. The average F_{ST} among the samples from the Maurienne valley (Sites 2–7 on Fig. 1) is lower but still indicates a significant population substructure ($F_{ST} = 0.031$, $P < 0.001$).

This suggests that genetic distance (measured by F_{ST}) increases with geographical distance as illustrated in Fig. 2(a,b). However, when a Mantel test is applied to all

data points (using either linear or valley distance, see Materials and methods) it is not significant. Close examination of Fig. 2 reveals that seven points at the bottom-right are outside the general trend and correspond to the seven pairwise comparisons involving Les Ecrins, the only sample outside the *Massif de la Vanoise*. When these points are not used the correlation becomes significant ($r = 0.497$, Mantel test: $P < 0.05$), even though the number of data points decreases. When the valley distance is used, the level of correlation is very similar but the test is not significant ($r = 0.484$, $P = 0.18$).

Discussion

Genetic variation within marmot populations and inbreeding avoidance

Reasonably high levels of genetic polymorphism were detected in the Alpine marmot using nuclear DNA microsatellite loci. The level of genetic diversity observed in Alpine marmots is similar to that observed microsatellite data sets in various mammals such as the mountain sheep (Forbes *et al*. 1995), the Mediterranean mouflon (Petit *et al*. 1997), wolves (Forbes & Boyd 1996, 1997), black bears and polar bears (Paetkau & Strobeck 1994; Paetkau *et al*. 1995), grey seals (Allen *et al*. 1995), or wild mice (Dallas *et al*. 1995; Blouin *et al*. 1996). However, one should be cautious in these comparisons since results from monomorphic loci are rarely published, and average levels of heterozygosity may vary much more across species than is apparent from published microsatellite data leading to a general overestimate of genetic variability. Our results contrast those published previously on *Marmota marmota* using minisatellites (Rassmann *et al*. 1994; $H_E = 0.12$) and microsatellite fingerprinting (Kruckenhauser *et al*. 1997). Genetic variation at allozyme loci was particularly low (≈1%) compared to other marmot or related species ($H_E = 0.053$–0.107, see references above). This led Preleuthner & Pinsker (1993) to hypothesize an ancient Pleistocene bottleneck while Rassmann *et al*. (1994) explained their results by a possible succession of harsh winters.

Our results do not suggest low levels of genetic variability in Alpine marmots of the French Alps. There are at least three possible explanations: (i) the six loci we used were particularly polymorphic by chance; (ii) the French populations of marmots have high diversity because of a different demographic history compared to Austrian and German populations; or (iii) the French, Austrian and German populations of marmots have similar demographic histories but more genetic diversity was maintained in the French populations by chance alone. In order to be able to differentiate between these hypotheses, we need to analyse populations from Austria and Germany with the six microsatellite loci used in this study. If we observe similar levels of genetic variation then this will indicate that by chance the mini- and microsatellites used by Rassmann *et al*. (1994) and Kruckenhauser *et al*. (1997), respectively, were not as polymorphic as ours. If we reject hypothesis one and demonstrate lower levels of diversity in Austria and Germany, it would be important to evaluate potential differences in the demographic histories of the populations.

There were no significant departures from HW proportions in the samples from the Maurienne valley, but both Les Ecrins and La Sassière showed strong and significant heterozygote deficits. These two samples are known to be a collection of well-defined family groups (14 within Les Ecrins and 18 within La Sassière), suggesting a possible Wahlund effect. This hypothesis was tested by evaluating differentiation between these family groups using F_{ST} and testing for HW proportions within each group. The average F_{ST} was high and significant ($F_{ST} = 0.28$, $P < 0.001$). All 153 pairwise F_{ST} values were significant within La Sassière (range: 0.02–0.41, Table 4) and 78 out of 91 were significant within Les Ecrins (range: 0.00–0.49). The 13 non significant (NS) values involved some of the smallest family groups in Les Ecrins ($3 < n < 7$), perhaps indicating a sample effect. Thus, the Wahlund effect seems the most reasonable factor explaining the observed heterozygote deficits (and the LD, as discussed above). This is also in good agreement with the fact that when Les Ecrins and La Sassière are removed there is no departure from HW equilibrium ($F_{IS} = 0.000$, NS) in the rest of the samples.

Other factors such as selection, assortative mating or null alleles could also contribute to the observed deficit in heterozygotes. These factors cannot be completely ruled out but are less likely explanations. If any of these factors were present we would expect to find heterozygote deficits in the family groups as well, whereas we find heterozygote excesses. Indeed, the F_{IS} values within the family groups are negative in 30 out of 32 family groups (14 being significantly different from zero and an average $F_{IS} = -0.16$, $P < 0.001$). Note that heterozygote excess is expected when the number of breeders is small (Pudovkin *et al*. 1996). However, this effect is usually small and cannot be responsible for the significant values observed because we used permutation methods.

Heterozygote excess could be caused by overdominance or by outbreeding. Because a heterozygote excess was observed at all loci, outbreeding is most likely. Also there is good evidence for outbreeding in some social species such as for the black-tailed prairie dog (Dobson *et al*. 1997). Negative F_{IS} values have been described within social groups in a certain number of species (e.g. Schwartz & Armitage 1980 for the yellow-bellied marmot; Foltz & Hoogland 1983 for the black-tailed prairie dog; Melnick *et al*. 1984 for rhesus monkeys) and have generally, though not necessarily, been interpreted as evidence for avoidance

Table 4 Pairwise F_{ST} (without SS-Bibl25) between family groups from La Sassière populations. NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

	A	B	C	D	E	F	G	I	J	K	L	N	O	P	R	U	Ch	La
A		0.146	0.135	0.229	0.140	0.023	0.165	0.247	0.172	0.229	0.184	0.143	0.150	0.213	0.287	0.187	0.114	0.252
B	***		0.091	0.214	0.130	0.114	0.243	0.292	0.207	0.170	0.150	0.254	0.135	0.251	0.269	0.189	0.178	0.293
C	***	***		0.280	0.210	0.075	0.348	0.326	0.265	0.254	0.171	0.240	0.162	0.305	0.307	0.285	0.074	0.293
D	***	***	***		0.119	0.205	0.176	0.245	0.302	0.353	0.100	0.249	0.196	0.152	0.353	0.237	0.310	0.357
E	***	***	***	***		0.133	0.181	0.202	0.189	0.244	0.163	0.141	0.129	0.139	0.294	0.068	0.206	0.187
F	*	***	***	***	***		0.218	0.229	0.175	0.221	0.137	0.116	0.122	0.210	0.246	0.182	0.050	0.181
G	***	***	***	***	***	***		0.260	0.246	0.306	0.239	0.296	0.246	0.182	0.380	0.250	0.397	0.410
I	***	***	***	***	***	***	***		0.198	0.249	0.237	0.176	0.149	0.048	0.333	0.159	0.314	0.361
J	***	***	***	***	***	***	***	***		0.117	0.194	0.221	0.154	0.143	0.221	0.124	0.257	0.189
K	***	***	***	***	***	***	***	***	**		0.169	0.284	0.118	0.203	0.231	0.168	0.272	0.349
L	***	***	***	**	**	***	***	***	***	**		0.217	0.065	0.127	0.147	0.189	0.212	0.271
N	***	***	***	***	***	***	***	***	***	***	***		0.090	0.174	0.292	0.153	0.153	0.213
O	***	***	***	***	***	***	***	***	***	***	*	***		0.111	0.108	0.122	0.145	0.242
P	***	***	***	***	***	***	***	***	***	***	***	***	***		0.260	0.084	0.297	0.295
R	***	***	***	***	***	***	***	***	***	**	**	***	**	***		0.308	0.330	0.315
U	***	***	***	***	*	***	***	***	**	***	***	**	***	**	***		0.293	0.275
Ch	**	***	*	***	***	*	***	***	***	***	***	***	***	***	***	***		0.320
La	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	

of inbreeding. Using allozymes, Foltz & Hoogland (1983) found a negative and significant $F_{IS} = -0.058$ within a single colony (several groups in patches) of the black-tailed prairie dog (*Cynomys ludovicianus*), and concluded that the species was relatively outbred. On the same species, Dobson *et al*. (1997) found that apparent inbreeding was caused by a Wahlund effect. Indeed, whenever information was available on existing substructure ('coteries') positive F_{IS} was explained by F_{ST} between these coteries, which themselves exhibited negative F_{IS} values. Depending on the geographical scale and the size of the colony analysed F_{IS} varies from 0.00 in their study to 0.09 in a study by Daley (1992) and 0.40 in a study by Chesser (1983). The conclusion of Dobson *et al*. (1997) was that as 'spatial scale is expanded from coterie to colony, to regional […] the rate of inbreeding […] changes from negative to zero, to positive.' A similar conclusion was made by Chesser (1991) who explained apparent positive F_{IS} values by inaccurate identification of coterie members. Schwartz & Armitage (1980) also obtained negative F_{IS} values of (−0.09) in yellow-bellied marmot (*M. flaviventris*) colonies, a result which they explained by an avoidance of consanguineous mating. All these results are thus very similar to ours where the positive values are observed on the two locations presenting the greatest amount of substructure while the smaller Maurienne valley samples exhibit values that can be either positive, negative or close to zero (Table 1) depending on the number of family groups which were sampled by chance. For Maurienne valley samples we do not have social structure data and thus cannot test whether F_{IS} increases with the number and differentiation of family groups. The results obtained in Les Ecrins and La Sassière and in other species are, however, good indications that this is probably the case, and that, in these social species, outbreeding rather than inbreeding is observed at the family group level. This may suggest the existence of behavioural mechanisms, which evolved to minimize inbreeding.

Behavioural mechanisms that can be invoked (Blouin & Blouin 1988; Pusey & Wolf 1996) are: (i) extra-pair or extra-group copulations; (ii) male-biased dispersal of both subadults and adults associated with strong philopatry of females; and (iii) avoidance of related individuals by oestrous females. Evidence for these behavioural mechanisms is evaluated below. The mating system usually defined for the Alpine marmot is monogamy (Zelenka 1965; Barash 1976; Arnold 1990a; Perrin *et al*. 1993), but Goossens *et al*. (1998a) have shown, on the basis of paternity exclusion analysis, that there is a high level of extra-pair paternity (≈30%) which could be the result of the presence of 'floater' or transient males rather than males from neighbouring groups. One could argue that the level of extra-pair copulation was overestimated because the previous dominant males died (predation, starvation, etc.) and were replaced. This could not be the case in the population studied by Goossens *et al*. (1998a) because dominant males of previous years were known and pedigree analysis were performed only for the family groups for which this information was available. Interestingly, Armitage (1974) also described this 'floater' strategy for

the yellow-bellied marmot (*M. flaviventris*). Such males would be adopting a strategy of peripheral living, waiting for an opportunity to mate and perhaps become resident. Consistent with this 'floater' strategy, Armitage (1974) found that none of the males replacing resident males in the study population were relatives. It is, however, difficult to determine whether these males are transient males that became resident or dispersing males from neighbouring populations (i.e. the second point).

Results concerning female philopatry and male-biased dispersal [point (ii)] have been more conflicting. In mammals, as opposed to birds, the dispersing sex is often the male (Greenwood 1980). This sex-biased dispersal has been observed in black-tailed prairie dogs by Dobson *et al*. (1997), who also note a strong female philopatry. Movements of males have also extensively been described in marmots. However, rarer movements involving young females have also been observed (Arnold 1990a,b; Magnolon 1999), indicating that in *M. marmota* sex-biased dispersal might exist without requiring a strict female philopatry. Interestingly, sex-biased dispersal is expected to be stronger in polygynous than in monogamous mammals (Dobson 1982). Recent genetic results based on measures of relatedness (B. Goossens *et al*., in preparation) indicate that males could be slightly less related to each other than females are among themselves. This confirms that males disperse slightly more than females but that strict female philopatry is an oversimplification of the Alpine marmot breeding system. There is no evidence for the third mechanism of inbreeding avoidance, i.e. avoidance of related individuals by females and our data do not allow us to either favour or reject it.

Genetic variation among populations

Given the geographical area surveyed, our results indicate a large amount of genetic differentiation with F_{ST} values between 0.02 and 0.26 [Nei (1978) classified $F_{ST} < 0.05$ low, 0.05–0.15 medium, >0.15 high]. For comparison, the average F_{ST} among greater horseshoe bat populations (Rossiter *et al*. 2000) between Italy and England are similar to those between Les Ecrins and some of the samples. Similar values are also observed in Switzerland in the greater white-toothed shrew, a very philopatric species (average $F_{ST} = 0.14$, Favre *et al*. 1997). This can be seen either between the samples within the Maurienne valley (populations 2–7, average $F_{ST} = 0.03$) or even between family groups within Les Ecrins and La Sassière (average $F_{ST} = 0.28$). Based on F_{ST} and IBD analyses, Les Ecrins is the most differentiated from all other samples and has the lowest amount of gene flow with other population in this study. The large F_{ST} are thus consistent with the hypothesis that marmot populations are relatively small (and thus subject to drift) and isolated from each other.

The pattern of genetic differentiation fits the isolation by distance model on a landscape scale. When Les Ecrins was removed from the data set, we found a significant correlation between geographical and genetic distances. This seems to indicate that IBD might be a reasonable model of dispersal only within the *Massif de la Vanoise* and that longer migration events might be rarer. Within this upland area the correlation was higher for linear than valley distance and not significant for the latter. This result is difficult to interpret. A direct interpretation of the results would be that marmots migrate in any direction and do not particularly favour movements along the valleys, as one would naively believe. Interestingly this is in agreement with field observations (L. Graziani, personal communication) where marmots have been seen crossing passes and glaciers at altitudes higher than 3000 m. At the same time we must be careful with this statistical analysis because the difference between the two results (valley vs. linear) is not very important. Our sampling scheme is not regular enough to avoid biases (only one sample comes from the Isère valley and six from the Maurienne valley). Also, the two measures of geographical distances are highly correlated as shown by a Mantel test ($r = 0.766$, $P < 0.05$) suggesting that even with large sample data sets it may not be easy to discriminate them.

The general pattern that arises from the genetic data is that the Alpine marmot is highly structured at the local level with large differentiation between family groups and populations. At a larger geographical scale IBD is observed indicating that migration is more likely between close or neighbouring populations. However, we cannot clearly decide whether migrations are more likely to occur along the valleys or in any direction. The results also confirm the idea that long distance migration events between upland areas are probably very rare. How rare is difficult to estimate.

Conclusions

The current study provided some new results on the Alpine marmots. It represents the first study that shows significant amount of genetic variability. As we suggested this may be caused by the fact that different regions of the Alps were sampled or by the fact that previously used hypervariable markers were not as polymorphic by chance. This study is also the first that analysed populations of marmots by taking into account the social structure within population and assessing inbreeding at different levels (region, valley, population, and family groups). We found that family groups are outbred rather than inbred. Interestingly the results we obtain are very similar to those obtained by other researchers on the black-tailed prairie dog for which a similar sampling strategy was observed. Our study clearly demonstrated that the sampling strategy can have dramatic effects on the results and interpretation

of the genetic data if behavioural information is not taken into account.

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References

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology*, **4**, 653–662.

Armitage KB (1974) Demography of yellow-bellied marmot populations. *Ecology*, **55**, 1233–1245.

Arnold W (1990a) The evolution of marmot sociality: I. Why disperse late? *Behavioral Ecology and Sociobiology*, **27**, 229–237.

Arnold W (1990b) The evolution of marmot sociality: II. Costs and benefits of joint hibernation. *Behavioral Ecology and Sociobiology*, **27**, 239–246.

Barash DP (1976) Social behaviour and individual differences in free-living alpine marmots (*Marmota marmota*). *Animal Behaviour*, **24**, 27–35.

Belkhir K, Borsa P, Chikhi L, Goudet J, Bonhomme F (1996–1997). *GENETIX 3.07, Windows™ Software for Population Genetics*. Laboratoire Génome et Populations, University of Montpellier II, Montpellier, France.

Blouin SF, Blouin M (1988) Inbreeding avoidance behaviors. *Trends in Ecology and Evolution*, **3**, 230–233.

Blouin MS, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology*, **5**, 393–401.

Burke T, Dolf G, Jeffreys AJ, Wolff R, eds (1991) *DNA Fingerprinting: Approaches and Applications*. Birkhäuser Verlag, Basel.

Chesser RK (1983) Genetic variability within and among populations of the black-tailed prairie dog. *Evolution*, **37**, 320–331.

Chesser RK (1991) Influence of gene flow and breeding tactics on gene diversity within populations. *Genetics*, **129**, 573–583.

Daley JG (1992) Population reductions and genetic variability in black-tailed prairie dogs. *Journal of Wildlife Management*, **56**, 212–220.

Dallas JF, Dod B, Boursot P, Prager EM, Bonhomme F (1995) Population subdivision and gene flow in Danish house mice. *Molecular Ecology*, **4**, 311–320.

Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour*, **30**, 1183–1192.

Dobson FS, Chesser RK, Hoogland JL, Sugg DW, Foltz DW (1997) Do black-tailed prairie dogs minimize inbreeding? *Evolution*, **51**, 970–978.

Ellegren H, Savolainen P, Rosén B (1996) The genetical history of an isolated population of the endangered grey wolf *Canis lupus*: a study of nuclear and mitochondrial polymorphisms. *Philosophical Transactions of the Royal Society of London B*, **351**, 1661–1669.

Epperson BK, Li TQ (1997) Gene dispersal and spatial genetic structure. *Evolution*, **51**, 672–681.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London B*, **264**, 127–132.

Foltz DW, Hoogland JL (1983) Genetic evidence of outbreeding in the black-tailed prairie dog (*Cynomys ludovicianus*). *Evolution*, **37**, 784–797.

Forbes SH, Boyd DK (1996) Genetic variation of naturally colonizing wolves in the central Rocky Mountains. *Conservation Biology*, **10**, 1082–1090.

Forbes SH, Boyd DK (1997) Genetic structure and migration in native and reintroduced Rocky Mountain wolf populations. *Conservation Biology*, **11**, 1226–1234.

Forbes SH, Hogg JT, Buchanan FC, Crawford AM, Allendorf FW (1995) Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. *Molecular Biology and Evolution*, **12**, 1106–1113.

Frey-Roos F (1999) *Geschlechtsspezifisches Abwanderungmuster beim Alpenmurmeltier (*Marmota marmota*)*. PhD Thesis. University of Marburg, Germany.

Garnier-Géré P, Dillmann C (1992) A computer program for testing pairwise linkage disequilibria in subdivided populations. *Journal of Heredity*, **83**, 239.

Goossens B, Coulon J, Allainé D *et al*. (1996) Immigration of a pregnant female in an alpine marmot family group: behavioural and genetic data. *Comptes Rendus de l'Académie des Sciences*, **319**, 241–246.

Goossens B, Graziani L, Waits LP *et al*. (1998a) Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA microsatellite analysis. *Behavioral Ecology and Sociobiology*, **43**, 281–288.

Goossens B, Waits LP, Taberlet P (1998b) Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. *Molecular Ecology*, **7**, 1237–1241.

Gottelli D, Sillero-Zubiri C, Applebaum GD *et al.* (1994) Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Molecular Ecology*, **3**, 301–312.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Hanken J, Sherman PW (1981) Multiple paternity in Belding's ground squirrel litters. *Science*, **212**, 351–353.

Houlden BA, England PR, Taylor AC, Greville WD, Sherwin WB (1996) Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. *Molecular Ecology*, **5**, 269–281.

Klinkicht M (1993) *Untersuchungen zum Paarungssystem des Alpenmurmeltiers*, Marmota m. marmota mittels *DNA fingerprinting*. PhD Thesis. University of Munich, Germany.

Kruckenhauser L, Miller WJ, Preleuthner M, Pinsker W (1997) Differentiation of Alpine marmot populations traced by DNA fingerprinting. *Journal of the Zoological Systematic and Evolution Research*, **35**, 143–149.

Magnolon S (1999) *La dispersion natale chez la marmotte alpine* (Marmota marmota). *Modalités et effets de quelques facteurs proximaux*. PhD Thesis. University of Tours, France.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Melnick DJ, Pearl MC, Richard AF (1984) Male migration and inbreeding avoidance in wild rhesus monkeys. *American Journal of Primatology*, **7**, 229–243.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Ohta T (1982) Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proceedings of the National Academy of Sciences of the USA*, **79**, 1940–1944.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.

Perrin C, Coulon J, Le Berre M (1993) Social behavior of alpine marmots (*Marmota marmota*): seasonal, group, and individual variability. *Canadian Journal of Zoology*, **71**, 1945–1953.

Petit E, Aulagnier S, Bon R, Dubois M, Crouau-Roy B (1997) Genetic structure of populations of the Mediterranean mouflon (*Ovis gmelini*). *Journal of Mammalogy*, **78**, 459–467.

Preleuthner M, Pinsker W (1993) Depauperated gene pools in *Marmota m. marmota* are caused by an ancient bottleneck: electrophoretic analysis of wild populations from Austria and Switzerland. *Acta Theriologica*, **38**, 121–139.

Preleuthner M, Pinsker W, Kruckenhauser L, Miller WJ, Prosl H (1995) Alpine marmots in Austria. The present population structure as a result of the postglacial distribution history. *Acta Theriologica*, **3**, 87–100.

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics*, **144**, 383–387.

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, **11**, 201–206.

Rassmann K, Arnold W, Tautz D (1994) Low genetic variability in a natural alpine marmot population (*Marmota marmota*, Sciuridae) revealed by DNA fingerprinting. *Molecular Ecology*, **3**, 347–353.

Rossiter SJ, Jones G, Ransome RD, Barratt EM (2000) Genetic variation and population structure in the endangered greater horseshoe bat *Rhinolophus ferrumequinum*. *Molecular Ecology*, **9**, 1131–1135.

Schwartz OA, Armitage KB (1980) Genetic variation in social mammals: the marmot model. *Science*, **207**, 665–667.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.

Spencer BS, Adams M, Marsh H, Miller DJ, Eldridge MDB (1997) High levels of genetic variability in an isolated colony of rock-wallabies (*Petrogale assimilis*): evidence from three classes of molecular markers. *Australian Journal of Zoology*, **45**, 199–210.

Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **3**, 277–290.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506–513.

Weir BS, Cockerham CC (1984) Estimating *F* statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**, 395–420.

Wright J, Tennant BC, May B (1987) Genetic variation between woodchuck populations with high and low prevalence rates of woodchuck hepatitis virus infection. *Journal of Wildlife Diseases*, **23**, 186–191.

Zelenka G (1965) Observations sur l'écologie de la marmotte des Alpes. *La Terre et la Vie*, **3**, 238–256.

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Appendix I

Analysis of linkage disequilibrium within Les Ecrins (without SS-Bibl25). NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NA = not applicable, for these pairs of loci, the absence of polymorphism does not allow the calculation of LD. The loci have been abbreviated to make the table more concise (example: 01/04 for SS-Bibl1/SS-Bibl4)

Loci	A1	A2	A4	A5	F1	F2	F3	F4	Ca	To	Pi	Go	Pa	U6
01/04	0.90	0	0.28	0	0	0.66	0.87	0.78	0.49	0.05	0.51	0	0.69	0.35
	NS	NA	NS	NS	NA	NS	NS	NS	NS	NS	NS	NA	NS	NS
01/18	0.56	0	0.19	0	0	0.96	0.39	0.43	0.09	0.31	0	0.37	0.16	0.13
	NS	NA	NS	NS	NA	NS	NS	NS	NS	NS	NA	NS	NS	NS
01/20	0.32	0	0.06	0	0	0.66	0.96	0.36	0.25	0.20	0.92	0	0.64	0.35
	NS	NA	NS	NS	NA	NS	NS	NS	NS	NS	NS	NA	NS	*
01/31	0.56	0	0.22	0	0	1	0.99	0.53	0.56	0	0.67	0	0.32	0.30
	NS	NA	NS	NS	NA	NS	NS	NS	NS	NA	NS	NA	NS	NS
04/18	0.66	0.49	0.34	0	0.51	0	0.96	0.34	0.13	0.44	0.77	0	0.47	0.31
	NS	NS	NS	NS	**	NA	NS	NS	NS	NS	NS	NA	NS	NS
04/20	0.96	0.45	0.20	0	0.22	1	0.96	0.50	0.39	0.73	0.45	0	0.56	0.28
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
04/31	0.66	0.16	0.38	0	0.10	0.44	0.58	0.55	0.34	0	0.27	0	0.42	0.35
	NS	NS	*	NS	NS	NS	NS	NS	NS	NA	NS	NA	NS	NS
18/20	0.77	0.38	0.31	0	0.21	0	0.99	0.50	0.30	0.49	0.94	0	0.32	0.30
	NS	NS	NS	NS	NS	NA	NS	NS	NS	NS	NS	NA	NS	NS
18/31	1	0.04	0.27	0	0.06	0	1	0.43	0.30	0	1	0	0.43	0.42
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA	NS	NA	NS	NS
20/31	0.77	0.51	0.38	0	0.23	0.44	0.99	0.49	0.69	0	0.98	0	0.81	0.15
	NS	NS	**	NS	NS	NS	NS	NS	NS	NA	NS	NA	NS	NS

Appendix II

Analysis of linkage disequilibrium within La Sassière (without SS-Bibl25). NS = non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NA = not applicable, for these pairs of loci, the absence of polymorphism does not allow the calculation of LD. The loci have been abbreviated to make the table more concise (example: 01/04 for SS-Bibl1/SS-Bibl4)

Loci	A	B	C	D	E	F	G	I	J	K	L	N	O	P	R	U	Ch	La
01/04	0.15	0.23	0.19	0	0.21	0.32	0.17	0	0.26	0.94	0.86	0.22	0.41	0.26	0.25	0.65	0.38	0
	NS	NS	NS	NA	NS	**	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NA
01/18	0.18	0.50	0.10	0	0.16	0.16	0.47	0.34	0.22	0.35	0.75	0.18	0.32	0.15	0.35	0.55	0.27	0.77
	NS	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
01/20	0.14	0.18	0.24	0.16	0.33	0.15	0.51	0.18	0.31	0.90	0.41	0.20	0.39	0.23	0	0.57	0.17	0.77
	NS	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
01/31	0.13	0.43	0.11	0.15	0.20	0.20	0.24	0.16	0.25	0.84	0.79	0.32	0.29	0.21	0.50	0.58	1	0
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
04/18	0.14	0.48	0.17	0	0.22	0.35	0.20	0	0.38	0.34	0.85	0.72S	0.42	0.23	0	0.36	0.47	0
	NS	**	NS	NA	NS	**	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NA
04/20	0.25	0.19	0.23	0	0.17	0.37	0.26	0.33	0.37	0.63	0.30	0.37	0.16	0.26	0	0.99	0.17	0
	NS	NS	NS	NA	NS	***	NS	NS	**	NS	NS	*	NS	NS	NS	NS	NS	NA
04/31	0.04	0.19	0.19	0	0.20	0.26	0.12	0.20	0.35	0.59	0.42	0.21	0.29	0.23	0.40	0.97	0.38	0
	NS	NS	NS	NA	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	*	NS	NA
18/20	0.22	0.17	0.25	0.09	0.34	0.33	0.51	0.22	0.42	0.97	0.30	0.30	0.24	0.18	0.42	0.46	0.53	1
	NS	NS	NS	NS	**	***	***	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS
18/31	0.16	0.29	0.94	0.43	0.28	0.34	0.61	0.08	0.26	0.98	0.47	0.31	0.31	0.12	0.31	0.23	0.27	0
	NS	NS	*	NS	NS	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
20/31	0.23	0.23	0.21	0.14	0.24	0.27	0.39	0.13	0.31	0.91	0.50	0.66	0.21	0.22	0.31	0.98	0.17	0
	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	**	NS	NS	NS	NS	NS	NS