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Microsatellite variation in autochthonous and introduced populations of the Alpine marmot (*Marmota marmota*) along a European west–east transect

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Abstract

Microsatellite variation was studied in 11 populations of the Alpine marmot along a west–east transect through the present distribution range. The samples represent five autochthonous and six introduced populations. Eleven loci were analysed in nine populations and six loci in the two populations from France. In the populations from the Western Alps, there is no indication for reduced variability as has been assumed in previous studies. However, a decrease of variation in the autochthonous populations was observed from the west to the east. The introduced populations showed a heterogeneous pattern reflecting the geographic origin of the released individuals. The population from the Spanish Pyrenees harbours a high level of variation and is genetically closest to the French populations. In Austria, three of the introduced populations have low variation and are closely related to the autochthonous populations from the western part of Austria. In contrast, two introduced populations from the central part of Austria are highly variable and resemble the populations from France. At least for one of these populations an early introduction of founder individuals from the Western Alps has been documented.

Key words: Microsatellites – heterozygosity – autochthonous populations – reintroductions – genetic differentiation – *Marmota marmota*

Introduction

The Alpine marmot (*Marmota marmota marmota,* Linné 1758) is a highly social rodent species of the sciurid family that is well adapted to open cold habitats (Arnold 1990a,b). As it is documented by the fossil record, the species occupied a much larger area during the Pleistocene, extending far to the south (northern Spain, Central Italy, Montenegro, Moldawia) and to the north (Belgium, Netherlands) (Preleuthner 1999; Preleuthner and Bauer 2001). However, forced by rising temperatures and the return of forests at the end of the ice age, marmots had to retreat to higher altitudes. In the course of this shift of the distribution range marmots have colonized the entire Alps where they are now restricted to a rather narrow zone above the tree-line at a preferred altitude of 1800–2200 m. In the lower regions of the Alps the range became fragmented when mountain massifs were separated by forests advancing along the valleys. Thus, some marmot populations inhabit mountain islands that are cut off from the core population of the higher Alps. These isolates are prone to extinction and in several areas populations may have vanished naturally when massifs of lower elevation were overgrown by forests.

Human activities have also strongly influenced the present distribution of Alpine marmots. First, overhunting and the use of marmots as an easily accessible source of food and fur has led to the successive extinction of local populations. This was especially the case in the eastern parts of the Austrian Alps east of the rivers Sill and Eisack where recolonization through natural dispersal was not possible. Although the presence of marmots in that region is documented in historical reports, most of these populations had probably disappeared until the middle of the 19th century. However, from 1860 on attempts were made to re-establish marmot populations. As a result of these reintroductions, which continued until the 1980s (Preleuthner et al. 1995), the eastern parts of the Austrian Alps have by now been recolonized and thus represent introduced populations. A similar situation is found in the Pyrenees where marmots had already disappeared about 15 000 years ago as a consequence of the climatic change (Herrero et al. 1999). Here, the introduction efforts started much later, in 1948, at the French side of the Pyrenees and were intensified in the 1960s and 1970s (Ramousse et al. 1993). Within a rather short period, stable populations developed in the French Pyrenees. From there marmots spread over the Spanish border and colonized the southern side of the Pyrenees through natural migration (Herrero et al. 1987, 1992).

Genetic investigations on Alpine marmots started with an extensive allozyme study carried out on the local population from Berchtesgaden (Germany), an autochthonous relict populations geographically separated from the Alpine core region. Surprisingly, genetic variation in this population turned out to be very low with only 4% (out of 50) polymorphic loci (Arnold 1990a). Using the same methods, Preleuthner and Pinsker (1993) analysed samples from 13 Austrian populations (both autochthonous and introduced). Their data indicated reduced variability at least in the eastern part of the distribution range. More recently, Bruns et al. (1999) detected a much higher degree of allozyme variation (17% polymorphic loci) in a large sample of autochthonous populations from Grisons (Switzerland) suggesting that variability may not be reduced in the more western populations.

Nevertheless, owing to their limited level of variation, allozymes proved unsatisfying as genetic markers for population and sociobiological studies in marmots, and therefore researchers switched to the hypervariable microsatellite loci. Rassmann et al. (1994) analysed the Berchtesgaden population using multilocus DNA fingerprinting and confirmed the low degree of polymorphism already revealed in the previous allozyme study (Arnold 1990a). Kruckenhauser et al. (1997) extended this investigation to six Austrian populations and two samples from Grisons (Switzerland). The results showed higher variability in the Swiss samples compared to five of the Austrian populations. The only exception was the introduced population from the Turracher Nockberge, a mountain range located in the south-central region of Austria, where variation

was equivalent to that of the Swiss samples. The first study on a marmot population from the Western Alps was attempted by Goossens et al. (2001), who analysed five microsatellite loci in samples from eight French locations. According to their data, the French marmots harbour a considerable amount of genetic variability similar to that found in various other mammals.

Summarizing the results of these earlier studies one could conjecture that a west–east decline of genetic variation may exist. However, the data obtained with different methods (allozymes, multilocus fingerprints, single locus microsatellites) are not directly comparable. Therefore, the present study employed a standardized method using a sufficient number (11) of microsatellite loci to investigate a set of samples that covers most of the current geographic range of the species. The samples include autochthonous populations from France, Switzerland and the western part of Austria as well as introduced populations from Austria and the Spanish Pyrenees. The aims of the study are to assess the amount of variation within and the degree of differentiation among the various populations and to discuss the differences in connection with introductions, possible founder events and geographic barriers.

Materials and Methods

Population samples

The origin, the status of the population (introduced or autochthonous), population code and numbers of investigated individuals per population are given in Table 1. Samples were provided by W. Arnold (Vienna, Austria), B. Goossens (London, UK), J. Herrero (Zaragoza, Spain) and M. Preleuthner (Vienna, Austria). The geographic locations of the sampling areas are depicted in Fig. 1. The samples from the Pyrenees (E/P; collection: 1994) were collected from two locations: Panticosa (eastern dot) and Echo (western dot of E/P in Fig. 1). The French sample from the Massif de la Vanoise (F/A; 1992–1996) comprises collections from four different locations (Chavière, Lans-le-Bourg, Lans-le-Villard and Sassière), which were pooled for the analyses because of the small size of the individual samples. The Swiss samples (CH/G; 1988 and 1998) consists of collections from three regions (Avers, Bivio and Val Fex) in the canton Grisons, which were also pooled. The Austrian samples (A/B, A/C, A/E, A/H, A/J, A/K, AM; 1985–1988) include two autochthonous populations from the western part and five introduced populations from the eastern part of Austria. For further details about the collection areas see Preleuthner (1993), Bruns et al. (1999), Herrero et al. (1999) and Goossens et al. (2001).

Table 1. Description of population samples

Geographic origin	Status	Code	*n*	Type	Source
A/Eisenerzer Alpen	int	A/M	20	Tissue	Preleuthner
A/Schladminger Tauern	int	A/K	14	Tissue	Preleuthner
A/Turracher Nockberge	int	A/J	19	Tissue	Preleuthner
A/Kreuzeckgruppe	int	A/H	26	Tissue	Preleuthner
A/Zillertaler Alpen	int	A/E	25	Tissue	Preleuthner
A/Verwallgruppe	aut	A/C	12	Tissue	Preleuthner
A/Lechquellengebirge	aut	A/B	22	Tissue	Preleuthner
CH/Grisons	aut	CH/G	45	Tissue	Arnold/ Preleuthner
F/Massif de la Vanoise	aut	F/A	34	DNA	Goossens
F/Les Ecrins	aut	F/B	9	DNA	Goossens
E/Pyrenees	int	E/P	8	Tissue	Herrero

Population status: int, introduced; aut, autochthonous; *n,* number of individuals. Country codes: A, Austria; CH, Switzerland; F, France; E, Spain.

DNA extraction

The samples from the French Alps consisted of DNA extracted from hair roots, which has been prepared and provided by Goossens et al. (1998). All other samples consisted of liver tissue obtained in the course of regular hunting activities. From liver samples approximately 100 mg tissue was frozen in liquid nitrogen, homogenized and dissolved in 1.4 ml extraction buffer (100 mM Tris–HCl, pH 8.0, 50 mM EDTA, 150 mM NaCl, 1% SDS). After adding proteinase K (100 μg/ml) the solution was incubated for 3 h at 50°C. The DNA was then purified by two phenol and one chloroform/isoamylalcohol (24:1) extractions.

PCR and fragment separation

The following 11 microsatellite loci were investigated: MS6, MS41, MS45, MS47, MS53, MS56 (Hanslik and Kruckenhauser 2000), Bibl4, Bibl18 (Klinkicht 1993), GS12, GS14, GS25 (Stevens et al. 1997). PCR amplifications were performed on a HYBAID Omnigene thermo cycler in a volume of 12.5 μl containing 10 mM Tris–HCl (pH 8.8), 1.5 mM $MgCl_2$, 150 mM KCl, 0.1% Triton X-100, 0.25 units DynaZyme DNA-polymerase (Finnzymes OY), 2 pM of each primer (forward primer labelled with IRD-800), 200 μM of each dNTP, 0.25 μl DMSO and 50 ng template DNA. The amplification protocols were as follows: 94°C 5 min (94°C 20 s, annealing temperature plus 6°C 20 s, 70°C 20 s) ×2; (94°C 30 s, annealing temperature 20 s, 70°C 20 s) ×27 (or ×30); 72°C 2 min. The annealing temperature was 53°C for MS6, MS41, MS45, MS53, MS56 and 50°C for MS47, Bibl4, Bibl18, GS12, GS14, GS25. PCR products were separated on 6% denaturating polyacrylamide gels in an LI-COR automatic sequencer. Analysis of PCR fragments was carried out with RFLPscan by Scanalytics.

Data analysis

Deviations from Hardy–Weinberg equilibrium were calculated for each of the 11 loci and a genotypic disequilibrium test was carried out with GENEPOP version 3.1d (Raymond and Rousset 1995). The same software was used to assess the genetic structure within populations by calculation of allele frequencies, average observed (H_0) and expected (H_E) heterozygosity, mean number of alleles per locus, probability of deviations from Hardy–Weinberg equilibrium and their significance (p), and the inbreeding coefficient (F_{IS}) (Weir and Cockerham 1984). The genetic distances between populations were estimated by Nei's standard distance, G_{ST} (Nei 1972), and R_{ST} (Slatkin 1995), which uses a high rate stepwise mutation model. Both distance matrices were calculated with the software MICROSAT 1.5 (Minch et al. 1996). G_{ST} distances were used to construct unrooted neighbour-joining trees with the software package PHYLIP version 3.57c (Felsenstein 1995). The trees were drawn with TREEVIEW version 1.6.5 (Page 1996) and adapted manually for the printed version.

Results

Amplification of microsatellites

In nine population samples all 11 microsatellite loci could be successfully amplified and genotyped. From the two French samples (F/A and F/B), reliable results could be obtained only for six loci (MS6, MS45, MS53, MS56, Bibl4 and Bibl18) owing to inferior quality of the available DNA extracts. Consequently, all calculations including data from these two French populations are based on six loci only. The allele frequencies are given in Table 2. All loci were variable with allele numbers ranging from three up to ten per locus. Five loci (MS6, Bibl14, Bibl18, GS12 and GS25) proved monomorphic in some populations. With the exception of allele 133 at locus GS12, all size differences were even numbered corresponding to the gain or loss of single or multiple dinucleotide repeats (Table 2).

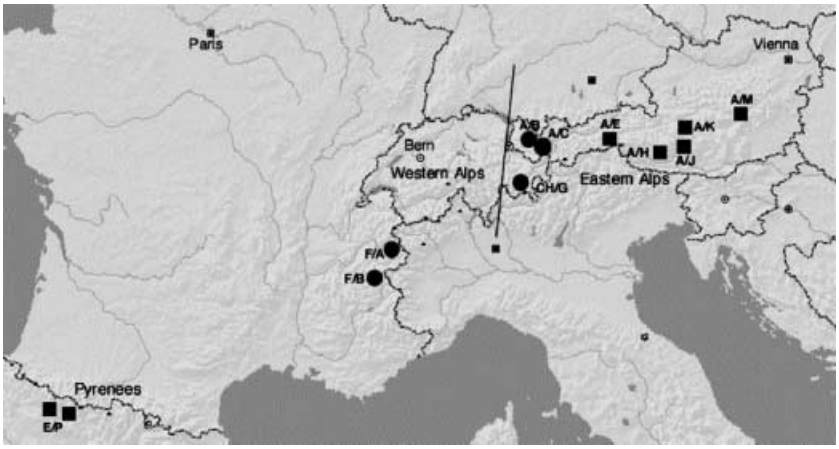

Fig. 1. Geographic distribution of the sampling areas. Autochthonous populations are shown as circles, introduced populations as squares. The borderline between Western and Eastern Alps is indicated. For area codes see Table 1

Null alleles and linkage

To account for the possibility of null alleles, the genotype distributions at the various loci were tested for deviations from Hardy–Weinberg equilibrium in each population. No deviations were observed with MS41. The other loci showed significant deficits of heterozygotes in one (MS45, MS53, MS56, Bibl18 and GS25), two (MS47, GS12 and GS14), three (MS6) or four (Bibl4) populations. Eight of 18 deviations from Hardy–Weinberg equilibrium were observed in the sample from Switzerland (CH/G). However, this may be ascribed to the fact that this population represents a pooled sample from three adjacent locations. A similar explanation can be assumed for MS6 where the deficit of heterozygotes occurred in the pooled samples CH/G and F/A, and in the very small sample E/P ($n = 8$). In contrast, at Bibl4 the deficit of heterozygotes was also detected in populations A/C and A/E, where the genotypes at the other loci matched the expected Hardy–Weinberg distribution. Therefore, the presence of null alleles has to be considered as a likely possibility for this particular locus only. To check the microsatellite loci for their independence, a test for genotypic disquilibrium was performed. Only two pairs of loci, Bibl18 and GS12 ($p \leq 0.05$) as well as MS47 and Bibl4 ($p \leq 0.005$), are possible candidates for linkage. As these disequilibria were only found in few populations we assumed that the loci were not physically linked and retained all loci for the analyses.

Genetic variation within the populations

Measures of genetic variation and population substructuring are summarized in Table 3. Among the autochthonous populations variation appears to be highest in the two western populations from France (F/A) and Switzerland (CH/G) compared to the populations from Austria (A/C, A/B). This is true for the average expected heterozygosity (H_E: 0.51–0.60 vs. 0.30–0.33) as well as the average number of alleles (n_a: 3.9–5.0 vs. 2.0–2.2). The second French population F/B shows lower values, which are closer to those of the Austrian populations. This may be the result of random effects caused by the small size of this sample and the fact that only six loci could be evaluated. The introduced populations show a heterogeneous pattern. In the Austrian populations, low values are found in the three populations A/M, A/H and A/E (H_E: 0.30, n_a: 1.8–2.9), whereas exceptionally high values were obtained for the adjacent populations A/K and A/J (H_E: 0.58–0.65, n_a: 3.5–4.1), similar to those of the most variable autochthonous population from France (F/A). The same is true for the Spanish sample from the Pyrenees (E/P) where, in spite of the small sample size, a high level of variation (H_E: 0.58, n_a: 3.6) is observed. The number of private alleles, that is, alleles occurring in one particular population only, is also highest in sample F/A from the French Alps, although only six loci have been analysed. This underlines the status of the French population as a genetic reserve for the gene pool of this species. In the Spanish population E/P, the number of private alleles was also high (four), but three of these alleles were found at loci that could not be studied in the French samples. It is likely that these alleles occur in the French populations too.

With respect to Hardy–Weinberg equilibrium only five populations showed no significant deviation at any locus: A/M, A/K, A/H, A/B and F/B (data not shown). These are also the populations with the lowest F_{IS} values (Table 3). In four populations (A/C, A/E, A/J and E/P), a deficit of heterozygotes was observed at one to three loci. The most striking departures were detected in populations CH/G (eight of 11 loci) and F/A (three of six loci). In both cases, the reason may be the pooling of samples from neighbouring locations. The data thus do not provide clear evidence for inbreeding within populations.

Genetic distances and relationships among populations

To assess the genetic differentiation among the 11 populations, R_{ST} values and Nei's standard genetic distances (G_{ST}) were calculated (Tables 4 and 5). The two parameters are in good agreement and the results obtained with either six or 11 loci are consistent. The G_{ST} values vary in the range of 0.05–1.46 (11 loci) or 0.07–3.27 (six loci), respectively. The pairwise R_{ST} values ranged from 0.01 to 0.56 (11 loci) and from 0.00 to 0.82 (six loci). With both parameters the lowest values are found among the samples from Austria whereas the highest values are observed in comparisons between Austrian and French or Spanish samples. From the G_{ST} values in Tables 4 and 5, unrooted neighbour-joining trees were constructed (Fig. 2a and b). Trees based on R_{ST} values had substantially the same topology (not shown). In both G_{ST} trees five of the Austrian populations, the autochthonous populations (A/B, A/C) together with three of the introduced populations (A/M, A/E and A/H), form a compact cluster

Table 2. Allele frequencies for the 11 microsatellite loci in 11 marmot populations

Locus	Allele	A/M	A/K	A/J	A/H	A/E	A/C	A/B	CH/G	F/A	F/B	E/P
MS6	142	–	0.393	0.028	–	–	–	–	0.044	0.038	0.333	0.125
	148	–	–	–	–	–	–	–	–	0.077	–	–
	150	–	–	–	–	–	–	–	–	0.135	–	–
	152	–	–	–	–	–	–	–	–	0.019	–	–
	154	–	–	–	–	–	–	–	–	0.019	–	–
	156	–	0.071	0.083	–	–	–	–	–	0.558	–	0.438
	158	–	–	–	–	–	–	–	0.178	0.154	0.611	0.188
	160	1.000	0.107	0.500	1.000	1.000	1.000	1.000	0.744	–	–	0.125
	162	–	0.429	0.111	–	–	–	–	0.033	–	0.056	0.125
	164	–	–	0.278	–	–	–	–	–	–	–	–
	2n	36	28	36	52	50	24	44	90	52	18	16
MS41	184	–	–	–	–	–	–	–	–	n.d.	n.d.	0.188
	186	0.467	0.385	0.735	0.692	0.891	0.458	0.318	0.833	n.d.	n.d.	0.625
	188	–	0.500	0.235	–	0.022	–	–	0.024	n.d.	n.d.	0.188
	190	0.533	0.115	0.029	0.308	0.087	0.542	0.682	0.143	n.d.	n.d.	–
	2n	30	26	34	52	46	24	44	84	–	–	16
MS45	109	–	0.143	0.344	–	–	–	–	0.081	0.206	0.556	0.313
	111	0.267	0.286	0.375	0.040	0.020	0.208	0.477	0.105	0.691	0.389	0.688
	113	0.733	0.571	0.281	0.960	0.980	0.792	0.523	0.814	0.103	0.056	–
	2n	30	28	32	50	50	24	44	86	68	18	16
MS47	163	–	0.107	0.094	–	–	–	–	–	n.d.	n.d.	–
	167	0.647	0.179	0.188	0.135	0.333	0.688	0.643	–	n.d.	n.d.	–
	173	–	–	–	–	0.042	–	–	0.110	n.d.	n.d.	–
	181	–	–	0.094	–	–	–	–	–	n.d.	n.d.	–
	183	–	–	–	–	–	–	–	0.024	n.d.	n.d.	0.063
	185	–	0.143	0.125	0.423	0.042	–	0.143	0.268	n.d.	n.d.	0.438
	187	–	0.143	0.094	–	0.042	0.062	–	0.488	n.d.	n.d.	0.500
	189	0.324	–	0.188	0.442	0.333	0.125	0.214	0.012	n.d.	n.d.	–
	191	0.029	0.429	0.219	–	0.188	0.125	–	0.098	n.d.	n.d.	–
	195	–	–	–	–	0.021	–	–	–	n.d.	n.d.	–
	2n	34	28	32	52	48	16	42	82	–	–	16
MS53	135	–	–	–	–	–	–	–	–	0.094	–	0.375
	141	–	0.143	0.389	–	–	–	–	0.011	0.266	0.222	0.188
	143	–	0.429	0.083	0.540	–	–	–	0.567	0.500	0.667	0.063
	145	0.053	–	0.139	0.300	0.220	0.250	0.024	0.189	0.109	0.111	0.188
	147	0.263	0.429	0.361	0.160	0.680	0.650	0.762	0.233	0.031	–	–
	149	0.684	–	0.028	–	0.040	0.100	0.214	–	–	–	0.188
	151	–	–	–	–	0.060	–	–	–	–	–	–
	2n	38	28	36	50	50	20	42	90	64	18	16
MS56	107	–	–	–	–	–	–	–	–	0.111	0.063	0.063
	109	–	0.071	–	–	–	–	–	–	0.130	0.813	0.125
	111	–	0.143	0.526	–	0.020	0.400	–	–	0.741	0.125	0.188
	113	0.225	0.714	0.395	0.327	0.320	0.600	0.190	0.956	0.019	–	0.625
	115	0.775	0.071	0.079	0.673	0.660	–	0.810	0.044	–	–	–
	2n	40	28	38	52	50	20	42	90	54	16	16
Bibl4	174	0.469	0.321	0.342	0.750	0.396	0.250	0.405	0.693	0.167	–	–
	176	–	–	–	–	–	0.100	–	–	0.021	–	–
	178	0.031	0.143	0.079	–	0.146	0.050	0.095	0.068	–	–	–
	182	–	–	0.079	–	–	–	–	–	–	–	–
	186	–	–	–	–	–	–	–	–	0.042	–	–
	188	–	0.036	0.184	–	–	–	0.024	–	0.375	–	0.125
	190	0.500	0.429	0.316	0.250	0.458	0.600	0.476	0.239	0.396	1.000	0.875
	192	–	0.071	–	–	–	–	–	–	–	–	–
	2n	32	28	38	48	48	20	42	88	48	8	16
Bibl18	127	–	–	–	–	–	–	–	–	–	–	0.125
	139	–	0.964	0.235	0.083	–	–	–	0.038	–	–	0.125
	141	–	–	–	0.125	0.022	–	–	–	0.022	–	–
	143	1.000	–	0.559	0.792	0.978	1.000	1.000	0.488	0.522	–	0.063
	145	–	–	0.147	–	–	–	–	0.438	0.196	1.000	0.250
	147	–	–	0.059	–	–	–	–	0.025	0.130	–	0.375
	149	–	0.036	–	–	–	–	–	0.013	0.065	–	–
	151	–	–	–	–	–	–	–	–	0.065	–	0.063
	2n	30	28	34	48	46	18	42	80	46	8	16
GS12	133	0.938	0.536	0.263	0.942	1.000	1.000	1.000	0.674	n.d.	n.d.	0.063
	142	–	0.214	0.632	0.058	–	–	–	0.174	n.d.	n.d.	0.063
	144	0.062	0.250	0.105	–	–	–	–	0.151	n.d.	n.d.	0.875
	2n	32	28	38	52	50	20	42	86	–	–	16
GS14	234	–	–	–	–	–	–	–	0.049	n.d.	n.d.	–

Table 2. (*Continued*)

Locus	Allele	A/M	A/K	A/J	A/H	A/E	A/C	A/B	CH/G	F/A	F/B	E/P
	242	–	–	–	–	0.042	–	–	0.024	n.d.	n.d.	–
	244	0.733	0.423	0.611	0.840	0.771	0.450	0.571	0.512	n.d.	n.d.	–
	246	0.267	0.269	0.194	0.160	0.188	0.550	0.429	0.415	n.d.	n.d.	0.250
	248	–	–	–	–	–	–	–	–	n.d.	n.d.	0.625
	252	–	–	–	–	–	–	–	–	n.d.	n.d.	0.125
	256	–	0.308	0.194	–	–	–	–	–	n.d.	n.d.	–
	2*n*	30	26	36	50	48	20	42	82	–	–	16
GS25	144	–	0.615	0.438	–	–	–	–	0.038	n.d.	n.d.	0.500
	146	–	0.038	0.344	–	–	–	–	–	n.d.	n.d.	0.312
	148	1.000	0.346	0.188	1.000	0.800	0.950	1.000	0.692	n.d.	n.d.	0.125
	150	–	–	0.031	–	0.120	0.050	–	0.231	n.d.	n.d.	0.063
	152	–	–	–	–	0.080	–	–	0.013	n.d.	n.d.	–
	158	–	–	–	–	–	–	–	0.026	n.d.	n.d.	–
	2*n*	28	26	32	50	50	20	42	78	–	–	16

$2n$ = number of microsatellites (per locus) examined. Allele designation relates to fragment size in bp. For populations F/A and F/B, only six loci could be analysed (n.d. = not determined).

Table 3. Average number of alleles per locus (n_a), number of private alleles over all loci examined (pA), observed (H_O) and expected (H_E) heterozygosity, and departures from Hardy–Weinberg equilibrium (F_{IS}) in 11 population samples

Population	n_a	pA	H_0	H_E	F_{IS}	p	2*n*
A/M	1.8	0	0.29	0.30	0.038	0.419	360
A/K	3.5	1	0.57	0.58	0.026	0.562	302
A/J	4.1	3	0.57	0.65	0.124	**	386
A/H	2.1	0	0.30	0.30	–0.004	0.946	556
A/E	2.9	2	0.20	0.30	0.342	***	536
A/C	2.2	0	0.22	0.33	0.337	*	226
A/B	2.0	0	0.28	0.30	0.062	0.556	468
CH/G	3.9	2	0.42	0.51	0.239	***	936
F/A	5.0	5	0.49	0.60	0.188	***	332
F/B	2.3	0	0.34	0.33	–0.034	0.620	86
E/P	3.6	4	0.42	0.58	0.282	0.131	176

Data are based on six loci in the two French (F/A, F/B) populations, and on 11 loci in all other populations. *p < 0.05, **p < 0.01, ***p < 0.001, $2n$ = number of microsatellites examined (over all loci).

Table 4. Genetic differentiation of nine marmot populations based on 11 microsatellite loci: above diagonal G_{ST} values, below diagonal R_{ST} values

	A/M	A/K	A/J	A/H	A/E	A/C	A/B	CH/G	E/P
A/M	–	0.718	0.465	0.109	0.082	0.084	0.035	0.326	1.271
A/K	0.360	–	0.330	0.586	0.594	0.680	0.694	0.401	0.588
A/J	0.445	0.223	–	0.420	0.352	0.457	0.472	0.341	0.533
A/H	0.299	0.346	0.423	–	0.073	0.154	0.148	0.158	1.340
A/E	0.165	0.385	0.462	0.164	–	0.090	0.095	0.212	1.276
A/C	0.012	0.352	0.445	0.281	0.168	–	0.052	0.290	1.462
A/B	0.012	0.376	0.457	0.323	0.248	0.020	–	0.362	1.289
CH/G	0.386	0.372	0.327	0.169	0.233	0.414	0.403	–	0.729
E/P	0.529	0.226	0.110	0.523	0.558	0.475	0.551	0.487	–

(G_{ST} values: 0.05–0.15 in Table 4). The Swiss population CH/G is associated to this cluster (average $G_{ST} = 0.27$) whereas the population from the Pyrenees (E/P) appears clearly separated by a long branch (Fig. 2a). Nevertheless, E/P is closer to the Swiss population ($G_{ST} = 0.73$) than to the Austrian cluster (average $G_{ST} = 1.33$), but the lowest distance values are found with respect to the Austrian populations A/K and A/J (average $G_{ST} = 0.56$). These two Austrian populations, which were also remarkable with respect to increased heterozygosity values, show a higher distance to the surrounding five populations than to the Swiss population (G_{ST} values: 0.65 vs. 0.40 for A/K, 0.43 vs. 0.34 for A/J). In the unrooted tree they are placed between the Swiss and the Spanish population. When the French samples are included (Fig. 2b), the topology of the tree remains the same for the populations from the Eastern Alps. The larger sample F/A is found between E/P and A/K, whereas the smaller sample F/B is rather distant to all the others (Table 5), but still closest to E/P ($G_{ST} = 0.45$) and F/A ($G_{ST} = 0.61$). Altogether, there seems to be a clear division between the Eastern Alps (Austria, Switzerland) and the populations from the Western Alps (France) and the Pyrenees (Spain). Only

Table 5. Genetic differentiation of 11 marmot populations (including two French samples) based on six microsatellite loci: above diagonal G_{ST} values, below diagonal R_{ST} values

	A/M	A/K	A/J	A/H	A/E	A/C	A/B	CH/G	F/A	F/B	E/P
A/M	–	0.980	0.389	0.138	0.085	0.146	0.058	0.387	1.086	1.846	1.060
A/K	0.415	–	0.435	0.714	0.793	0.930	0.893	0.452	0.989	1.037	0.591
A/J	0.404	0.244	–	0.402	0.337	0.325	0.309	0.341	0.339	0.984	0.457
A/H	0.393	0.367	0.359	–	0.092	0.163	0.179	0.165	1.071	1.744	1.453
A/E	0.078	0.405	0.396	0.243	–	0.070	0.056	0.283	1.235	2.031	1.315
A/C	0.065	0.373	0.345	0.298	0.000	–	0.098	0.341	1.067	3.271	1.446
A/B	0.023	0.395	0.338	0.348	0.110	0.048	–	0.427	0.972	1.777	1.044
CH/G	0.412	0.397	0.247	0.175	0.288	0.362	0.354	–	0.987	1.040	0.819
F/A	0.686	0.402	0.301	0.640	0.662	0.691	0.651	0.470	–	0.607	0.359
F/B	0.791	0.547	0.507	0.822	0.815	0.747	0.788	0.785	0.149	–	0.446
E/P	0.442	0.180	0.112	0.419	0.456	0.343	0.409	0.404	0.068	0.115	–

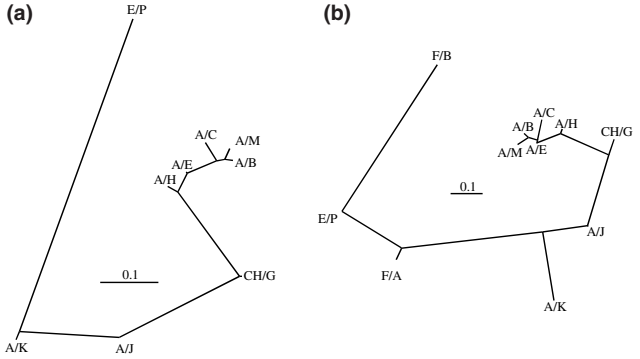

Fig. 2. Neighbour-joining trees based on G_{ST} values obtained from microsatellite data: (a) tree of nine populations analysed at 11 loci; (b) tree of 11 populations (including the French samples F/A and F/B) analysed at six loci

two of the introduced Austrian populations (A/K and A/J) do not fit into this phylogeographic scheme.

Discussion

The results clearly demonstrate that, as a species, the Alpine marmot is not genetically deprived. Among the autochthonous populations, the French marmots from the Massif de la Vanoise (F/A) showed the highest level of variation ($H_E = 0.60$). Three additional loci, which have been analysed by Goossens et al. (2001) but were not used in the present study, are in accordance with our data (average $H_E = 0.72$). Further to the east, in the autochthonous populations from Switzerland ($H_E = 0.51$) and Austria ($H_E = 0.30$–0.33), variability is decreasing. Even if the data of the three autochthonous samples from Austria are pooled in order to account for the fact that the French and Swiss samples consist of different subsamples from the respective areas, the decrease in variation is still obvious. The number of alleles detected at six loci is 31 in France (F/A + F/B), 21 in Switzerland (CH/G) and 15 in Austria (A/B + A/C). For 11 loci the respective numbers are 42 for Switzerland and 27 for Austria. The lower variation in the autochthonous populations from Western Austria could have been brought about by a historical bottle-neck that may have occurred in the course of or after the colonization of the Eastern Alps. Another possibility would be that genetically differentiated marmot populations had already developed during the ice age, which later colonized the Alps through different migration routes. In both cases the intermediate position of the Swiss population could be explained by gene flow from the Western Alps.

In the introduced populations, the genetic status is expected to reflect the geographic origin of the released individuals. In addition, founder effects and genetic drift in the developing populations may also have shaped the gene pool by reducing the initial amount of variation. In the Pyrenees, the introduced marmots came from the national parks La Vanois and Mercantour in the French Alps (Ramousse et al. 1993). This explains the close relationship to the autochthonous French populations F/A and F/B (Table 5). Furthermore, with more than 500 marmots successively released in the French Pyrenees, the number of founder individuals was quite big. Consequently, no reduction of variability can be observed and the heterozygosity level is still as high as in the French source population. At four loci (Bibl4, Bibl18, MS53 and MS56), the allele frequencies in population E/P differ significantly ($p < 0.001$) from the French sample F/A. In spite of the small sample size, this may indicate some restructuring of the gene pool by genetic drift or selective forces imposed by the new habitat. Furthermore, no samples were available from the second source population (Mercantour national park).

In Austria, the introductions were carried out in a different way. Preleuthner (1993) documented 119 individual introductions in the Austrian Alps, most of them performed by local hunters. In addition, an unknown number of unauthorized introductions may have occurred. The number of marmots released with each particular introduction was usually quite small (five individuals on the average), sometimes only a single pair. Very often the founder individuals were taken from non-autochthonous populations originating from previous introductions. As a consequence, some of these populations were exposed to repeated bottle-necks. Therefore, although the total number of marmots released over a period of 140 years can be estimated as more than 600, the effective population size was certainly much smaller.

The introduced Austrian populations analysed in the present study fall into two distinct categories: the genetically less variable populations A/E, A/H and A/M, and the highly variable populations A/J and A/K. Populations A/H (Eisenerzer Alpen) and A/M (Kreuzeckgruppe) inhabit isolated mountain massifs. In both cases some of the released marmots were from the province Tyrol, which would explain the close relationship to populations A/B and A/C from western Austria. Population A/E comes from a region adjacent to the autochthonous populations of western Tyrol, which is

separated by the Brenner pass. Because of the rather low altitude (1370 m), this possible migration barrier apparently prevented the spread of the autochthonous populations towards the east before the onset of the introduction efforts. Moreover, the presence of six alleles (Table 2) in A/E, which are not found in A/B or in A/C, suggests that gene flow in the opposite direction, from introduced to the autochthonous populations, is at least very slow. Four of the six alleles occur also in the Swiss population CH/G, indicating that the founders of A/E may have originated from that area.

Populations A/J and A/K are closest to each other (Table 4) and quite distinct from the other Austrian populations, both in genetic distance and heterozygosity levels. In the unrooted trees (Fig. 2a and b) they are associated with the western populations from France and Switzerland. This similarity is underlined by the presence of 14 alleles that were only detected in the western populations (E/P, F/A, F/B and CH/G) but in none of the other samples from Austria. Eight of these 'western alleles' are shared by A/J and A/K. Four introductions that could have contributed to population A/J are listed in Preleuthner (1993), but the details about origin and number of the released marmots are not further specified. For population A/K at least 10 introductions in that area are documented, mostly with marmots from the province of Tyrol. However, for one of the earliest introductions carried out in 1879 the origin is indicated as 'Western Alps', which could mean anything between France and the central part of Switzerland. As the alleles from this western source population seem to be still overrepresented in A/K, the contribution of later introductions to the current gene pool was apparently of minor importance. Populations A/K (Schladminger Tauern) and A/J (Turracher Nockberge) are not isolated by deep valleys. There is a connection over the Radstädter Tauern pass (1739 m) and Katschberg pass (1641 m) and it seems possible that marmots are able to migrate between the two areas. Accordingly, one could assume that gene flow of 'western alleles' into population A/J led to the present genetic similarity. However, it cannot be ruled out that parallel introductions of marmots from the Western Alps have occurred in A/J that have not been documented.

Another hypothesis to be taken into account is the possibility that autochthonous populations had never become completely extinct in the region of the Schladminger Tauern, which includes the area of population A/K (Preleuthner and Bauer 2001). Private alleles not found in other populations could be considered as remnants of a local autochthonous gene pool corroborating this hypothesis. Among the 48 different alleles recorded for populations A/J and A/K, five were not detected in any of the other samples (Table 2). However, two of these were alleles of loci that could not be analysed in the French samples and thus might be present in the French population. Whether the remaining three alleles are of ancient origin cannot be decided. At least there seems to be no strong support for the genetic contribution of an autochthonous relic population.

With respect to the genetic management of marmot populations, the present results show no indication of reduced genetic variability in the autochthonous populations of France and Switzerland. However, in some of the introduced populations of Austria the genetic variation appears to be low. This can be caused by the choice of the source population, the number of individuals released in the particular area and lack of gene flow from adjacent populations. As a practical consequence, if new introductions are to be carried out, attention should be paid to maintain the genetic variation. Founder effects, as they have occurred in some regions of the Austrian Alps, should be avoided, especially in isolated mountain massifs where the lost variation cannot be replenished naturally. The introduction of marmots in the Pyrenees shows that the selection of an appropriate source population may be the most important factor for establishing an enriched gene pool.

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Zusammenfassung

Mikrosatellitenvariation in autochthonen and eingebürgerten Populationen des Alpenmurmeltieres (Marmota marmota) entlang eines europäischen west-ost Gradienten

Die Mikrosatellitenvariation wurde in 11 Populationen des Alpenmurmeltieres entlang eines west-ost Gradienten durch das gegenwärtige Verbreitungsgebiet untersucht. Die Stichproben repräsentieren fünf autochthone und sechs eingebürgerte Populationen. Elf Loci wurden in neun Populationen analysiert und sechs Loci in den beiden Populationen aus Frankreich. In den Populationen aus den Westalpen ergab sich im Gegensatz zu früheren Untersuchungen kein Hinweis auf eine Reduktion der Variabilität. Innerhalb der autochthonen Populationen ist jedoch eine Abnahme der Variation von Westen nach Osten festzustellen. Die eingebürgerten Populationen zeigten ein uneinheitliches Muster, welches den geografischen Ursprung der ausgesetzten Individuen widerspiegelt. Die Population aus den spanischen Pyrenäen besitzt eine hohe Variation und ist genetisch den französischen Populationen am ähnlichsten. In Österreich zeigen drei der eingebürgerten Populationen niedrige Variation und sind den autochthonen Populationen aus Westösterreich am nächsten verwandt. Hingegen sind die beiden eingebürgerten Populationen aus Zentralösterreich hoch variabel und den Populationen aus Frankreich ähnlich. Zumindest für eine dieser beiden Populationen ist eine frühe Einbürgerung von Gründerindividuen aus den Westalpen dokumentiert.

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