Alpine marmots in Austria. The present population structure as a result of the postglacial distribution history

Monika PRELEUTHNER*, Wilhelm PINSKER, Luise KRUCKENHAUSER, Wolfgang J. MILLER and Heinrich PROSL


The present distribution of the Alpine marmot Marmota m. marmota (Linnaeus, 1758) in Austria, the historical range during the Pleistocene, and recent efforts of re-introduction were documented. Autochthonous populations inhabit a continuous range extending over the western part of the Austrian Alps. Non-autochthonous populations occur in a more fragmented area of partly isolated massifs in the east. The non-autochthonous populations were generated by the release of only a few founder individuals (median = 5). The impact of founder effects and migration barriers on the structure of the disjunct non-autochthonous populations is confirmed by the genetic analysis of allozymes and VNTR-loci. Whereas autochthonous populations are characterized by high genetic similarity and common polymorphisms, the non-autochthonous populations exhibit a more patchy pattern of variation caused by founder effects and subsequent drift. From the relationships indicated by the VNTR-patterns it appears possible to infer the putative origin of the founder individuals. In previous allozyme studies the genetic variability was found to be reduced with the exception of two widespread polymorphisms at the loci Pep-1 and Sod-1. A parasitological survey shows that Pep-1 genotypes differ in their degree of infestation by endoparasites (Citellina alpina and Ctenotaenia marmotae) indicating that this polymorphism may be maintained by selective forces.

Institut für Wildbiologie und Jagdwirtschaft, Peter Jordan Straße 76, A-1190 Vienna, Austria (MP); Institut für Allgemeine Biologie, AG Genetik, Währingerstr. 17, A-1090 Vienna, Austria (WP, LK, WJM); Institut für Allgemeine Zoologie und Parasitologie, Linke Bahng. 11, A-1030 Vienna, Austria (HP)

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Introduction

Genetic studies based on the electrophoretic analysis of enzymes have revealed a remarkably low degree of variation in wild populations of the Alpine marmot Marmota m. marmota (Linnaeus, 1758) (Arnold 1990, Preleuthner and Pinsker

* Present address: Konrad-Lorenz-Institut für Vergleichende Verhaltensforschung, Savoyenstr. 1a, A-1160 Vienna, Austria, to whom reprint requests should be sent.
1993). Although samples of the Alpine marmot from three different countries (Austria, Germany and Switzerland) have been included in these surveys, the pattern was nevertheless uniform. Only two out of fifty loci, *Pep-1* and *Sod-1*, were found polymorphic. Moreover, even these two genes displayed a rather constant polymorphism with two coexisting alleles present in most of the populations. In spite of the large sample size (2819 genes were screened for *Pep-1* and *Sod-1*), no additional rare variants beside the two common alleles could be detected. Especially the lack of rare alleles, together with the overall reduced level of variability, led to the conclusion that the gene pool of the entire species may have experienced a severe bottleneck. The rather high levels of polymorphism found in related species of the same genus (Schwartz and Armitage 1980, 1981, Wright et al. 1987) confirm the bottleneck hypothesis.

One aim of the present work was a detailed investigation of the postglacial distribution history of Alpine marmots in Austria. The present population structure can be understood as a result of different events: long term changes in the environment, eg climatic shifts, and the more dramatic influence through human activities, eg local extinction by overhunting or efforts to reestablish populations in particular areas (Preleuthner 1993). Three sources of information were evaluated for our approach to reconstruct the recent evolutionary history of the marmot populations. The distribution of fossil remains provides a rough idea about the distribution range of marmots during the Pleistocene (eg Mottl 1958, Bauer et al. 1979, Rabeder and Mais 1985, Fladerer 1989, Kunst et al. 1989, Rabeder 1991). Historic accounts about the occurrence of marmots in different areas of the Alps allow to demarcate the regions which have been inhabited by marmots before the onset of severe human influence (Zimmeter 1886, Dalla Torre 1887). Finally, in a census involving local hunting authorities the present geographic distribution was recorded, as well as any colonization efforts carried out during this century (Preleuthner 1993, and references therein).

Beside tracing the history of distribution, we also attempted to collect additional data on the present genetic structure of the gene pool. Allozyme variation is based on substitutions in the coding regions of structural genes. Assuming a drastic decrease in variation, polymorphism is only slowly restored by point mutations. Therefore the level of variation, once reduced by a bottleneck, remains low for a rather long period of time. In contrast, variability at VNTR-loci (variable number of tandem repeats) is expected to behave in a different way (Jeffreys et al. 1985, Tautz 1989). Although reduction in population number results in the loss of existing polymorphisms, new variation is generated by replication slippage and unequal crossing over (Armour and Jeffreys 1992). This mechanism is entirely different from that giving rise to amino acid replacements in enzymes, and, more important, has a considerably faster rate (Schlötterer and Tautz 1993, Charlesworth et al. 1994, Di Rienzo et al. 1994). Thus VNTR-loci should quickly reestablish the reduced variation. Due to the high variability VNTR-loci are considered to be excellent markers for population studies on otherwise genetically deprived species.
Genetics and distribution of *M. marmota* (Kuhnlein et al. 1989, Gilbert et al. 1990, Bruford et al. 1992). For the marmots they could be used to estimate the divergence of populations and to determine the possible origin of colonizers.

The widespread polymorphisms at the two enzyme loci Pep-1 and Sod-1 have apparently been maintained in spite of the postulated bottleneck and successive drift effects in local populations. This suggests that these polymorphisms might be balanced by selective forces (Preleuthner and Pinsker 1993). One possible factor influencing the fitness of marmots is the infestation by endoparasites. Therefore we examined a possible role of parasitism on the allele distribution at Pep-1 and Sod-1 by determining the genotypes of individual marmots through electrophoretic analysis and correlating the data with the results from the parasitological analysis of the intestinal tracts. Taking together the historic information, the data obtained at different levels of genetic analysis, and the parasitological investigation, we expected to get new insights into the forces shaping the genetic structure of a species exposed to extreme reductions of the population size.

**Material and methods**

**Distribution survey**

The present distribution of marmots in Austria was investigated by a census carried out through 1990–1991. The area of investigation covers the entire mountainous region of Austria. The topographic map OK 1:300,000 was divided into 8535 grid cells of 2.5 × 3.7 km. Map sheets of the particular areas and questionnaires were sent to local hunters. For each grid cell the occurrence or absence of marmots was recorded. The geographic information system software McGIS was used for the computer aided mapping (Preleuthner and Grinner 1990). In order to assess the historic range of marmots in Austria, 38 excavation sites of fossil remains were evaluated. In addition, historic reports about the occurrence of marmots were extracted from the literature. A compilation of the sources of information and references is given by Preleuthner (1993). Re-introduction efforts were recorded through the questionnaires from hunting authorities; 119 re-introductions were documented. For each particular case data were collected about the year, locality, number of individuals, and provenance of the released marmots. A detailed account is found in Preleuthner (1993).

**Population samples**

Sampling procedures and characterization of the sampling areas have been described in Preleuthner and Pinsker (1993). The area codes used in the present study relate to this previous report. For the parasitological analysis of the degree of infestation, two additional samples of non-autochthonous populations from Eastern Tyrol were evaluated: Innervillgraten (Iv) and Zetttersfeld-Deband-Tal (Zd). Thus the parasitological survey comprised 162 individuals from the following 12 populations: 3 autochthonous populations from Switzerland (A) and Austria (B, D), and 9 non-autochthonous populations from Austria (E, F, G, H, I, J, K, N, Iv, Zd). The genotypes at the loci Pep-1 and Sod-1 were determined in a pooled sample of 93 individuals.

The analysis of VNTR-loci was carried out on 99 marmots from 8 populations. Samples were taken from 4 autochthonous populations (Ab, Ag, B, and C), and from 4 non-autochthonous populations (E, H, J, M). The pooled sample from Switzerland originally designated A (Western Alps) by Preleuthner and Pinsker (1993) turned out to be genetically heterogenous in the VNTR-study. Because of the clear differentiation at the DNA-level, sample A was split into the two distinct subsamples Bern (Ab) and Graubünden (Ag).
Parasitology and allozyme study

The material used for this study was obtained as a by-product of regular hunting. It has to be emphasized that no marmots were killed for scientific purposes. The intestinal tracts of freshly shot marmots were removed and stored at −25°C for investigation. Four sections (stomach, small intestine, caecum, and colon) were examined separately for endoparasites according to the method given by Boch and Supperer (1983). The parasites were determined taxonomically and the degree of infestation recorded for each species separately (number of parasites). Parallel to the parasitological survey the genotypes of the marmots at Pep-1 and Sod-1 were investigated from liver samples. Horizontal starch gel electrophoresis was used for the analysis of the enzymes. Electrophoretic techniques and staining procedures were the same as in Preleuthner and Pinsker (1993).

DNA fingerprinting

Genomic DNA was extracted from deep frozen liver samples. From each sample 5 μg of DNA was digested with Hin f I. The restriction fragments were separated on 0.8% agarose gels at 30 V for 36 h. After Southern transfer, the nylon membranes were hybridized at 38°C with the DNA-probe (ATCC)4 labelled with fluorescine-11-dUTP (Amersham ECL™ 3'-oligolabelling kit). Washes were done four times (15 minutes each) at room temperature in 5 x SSC, 0.1% SDS. Detection was carried out by using anti-fluorescin HRP conjugate following manufacture's instructions (Amersham ECL™ Detection kit). Lambda-DNA digested with Hin d III (Boehringer Mannheim) was used on either side of the gels as a molecular weight maker. All bands corresponding to fragments from 4 to 23 kb were scored. Bands of similar molecular weight and intensity of the signal were recorded as identical.

Results

Present distribution of marmots

The current distribution of Alpine marmots in Austria monitored in the 1990/1991 census is shown in Fig. 1. For each grid cell of the map local hunters...
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recorded independently whether the area is inhabited by marmots or not. It was not attempted to determine the number of individuals living in particular areas, since even rough estimates of the population size have turned out to be not reliable. Thus the map in Fig. 1 gives a valid account of the extension of the distribution range, but does not allow any direct inferences about population densities. According to this investigation, the present distribution range covers the entire mountain chain of the Alps in Austria. Only a few isolated massifs at the northern (Tyrol, Salzburg, Upper Austria) and southern (Carinthia) margins of the Alps are spared out. Marmots are found at altitudes between 900 m and 2700 m a.s.l. Hunting statistics indicate, however, a preference for the upper subalpine zone (1800–2200 m). In the western part of Austria the distribution range is more or less continuous, interrupted only by one deep valley in Tyrol cut by the rivers Sill and Eisack (Wipptal). This valley separates the western from the eastern populations and probably prevents migration between these two areas. A more patchy distribution is observed in the eastern part of Austria. Although connected along the main crest of the Alps, the distribution range has a ragged appearance. Several detached populations inhabit isolated massifs, especially in the northern and southern limestone ranges. It can be assumed that most of the area providing suitable habitats for marmots in Austria is now populated by this species.

**History of distribution**

The present distribution range is the product of a historical process which started at the end of the last glaciation. In an attempt to assess the distribution of marmots during the Pleistocene, the geographic locations of 38 excavation sites

![Fig. 2. Re-introduction efforts in Austria. The locations where marmots have been released are marked by dots. Massifs inhabited by autochthonous populations are indicated by dark shading, massifs inhabited by re-introduced populations are indicated by light shading. Dots outside the map represent re-introductions where only the province is known but not the location of the release.](image-url)
containing fossil remains of marmots were recorded (for details see Preleuthner 1993). The sites are located at altitudes between 275 m and 2800 m a.s.l., the majority of them outside or along the margins of the present range (Fig. 2). Most of the fossils were recovered from caves (30 sites). With respect to the geological age, most of them can be attributed to the Upper Pleistocene, only the remains of two sites are older: one from the Middle Pleistocene (Mottl 1958, Bauer et al. 1979) and one from the Lower Pleistocene (Scharfe 1989). The latter one has been identified as M. primigenia, the ancestor of the recent species.

The fossil data indicate that during the last glaciation marmots inhabited a wide area around the present range including the plains of central Europe, which were devoid of forest over this period. With rising temperatures, however, the advancing forest forced the marmot populations to recede to the higher altitudes of the present area. It is likely that in the course of this retreat into the Alpine refuges marmots have colonized the entire region of the inner Alps. In some areas, however, the rising timberline reached the summits of the mountains and thus led to local extinction of the marmot populations. In other regions marmots were probably wiped out by human activities. Historical reports, summarized by Preleuthner (1993), document the former occurrence of marmots in some of these areas. Until about 1800 the populations in the eastern part of Austria (east of Sill and Eisack) had almost completely vanished. Because of the discontinuity of the potential habitats, restoration of the population through natural migration from adjacent massifs was inhibited. Only in the area around Berchtesgaden (Germany) protective measures imposed by the local government prevented marmots from getting exterminated. In contrast, the western part of Austria was continuously occupied by autochthonous marmot populations. Although in this region local populations may have become also severely reduced from time to time,

Fig. 3. Number of individuals released. Most re-introductions were carried out with a small number of founder individuals (median = 5).
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they were probably never endangered. In the large and compact Alpine area of western Austria the lack of natural barriers allowed free exchange of individuals and thus any decrease in population number was easily replenished.

From about 1860 attempts were made to reestablish marmot populations in the eastern mountain ranges. In the course of these activities, marmots were successively released by local hunters. In our survey 119 re-introduction efforts could be documented. The re-introductions started around 1860 and continued until a first peak was reached before the outbreak of World War I. Due to the wars and the economic depression the activities succumbed during the following decades. From 1950, however, the re-introduction efforts were resumed and even intensified over the last years. A detailed account of the re-introductions is given by Preleuthner (1993). The number of marmots released in each particular area was quite low (Fig. 3), ranging from 2 to 16, with a median of only 5 individuals. Nevertheless, most of the re-introductions proved successful and stable populations developed quickly. Very often the founders were taken from non-autochthonous populations originating from previous re-introductions with already small numbers of individuals. Thus some of the non-autochthonous populations were exposed to repeated bottlenecks.

Variation at VNTR-loci

Because enzyme loci had failed to provide enough variable markers for investigating the genetic differentiation among populations, a fingerprint analysis was carried out using the DNA-probe (ATCC). This probe has already been tested by Rassmann *et al.* (1994) and successfully employed for studying the relationships between individual marmots in a local population from Berchtesgaden (Germany). Ten variable bands (23.1, 20.0, 18.3, 14.5, 12.5, 7.0, 5.8, 5.4, 4.8, 4.4 kb) were distinguished, consecutively numbered from 1 to 10 according to their fragment size. The number of bands per individual ranged from two to six. 32 different pattern-types (combinations of bands) were observed among the 99 individuals from eight populations. Examples are given in Fig. 4. These pattern-types have to be considered as phenotypes. To resolve the genetic basis of the pattern types would require the analysis of pedigrees with secured relationships of the individuals. This was of course not possible with the available material. Thus we do not know how many loci are involved and which bands represent allelic variants of the same locus.

In spite of these shortcomings the geographic distribution of the different bands provides useful information about the relationships among the populations. The relative frequencies of the ten different bands were determined for each of the eight population samples. The frequency of a particular band was divided by the total of bands recorded in the respective population (Fig. 5). No difference could be made between homozygous and heterozygous bands. Therefore the frequencies obtained in this way do not represent allele frequencies. Nevertheless, a comparison among the populations on the basis of band frequencies allows at least a rough estimation of genetic relationships.
Fig. 4. Example of a DNA fingerprint obtained with the probe (ATCC)k. Six of the ten variable bands, indicated by arrows, can be seen on this blot. The size of the fragments is given in kb. Pattern types t-2, t-3, t-4, t-5, and t-7 are common types found in several populations. In contrast, the rare type represents a pattern observed only in one of the eight population samples. S – standard control lane loaded with a mixed sample of defined genotypes.

All eight populations were polymorphic, the number of different bands, however, varied between five (population M) and ten (population J). The differentiation of the autochthonous populations reflects gradual differentiation due to inhibited gene flow between partly isolated areas. The autochthonous populations from Austria (B, C) are genetically closely related. Both are clearly distinct from the westernmost Swiss population (Ab). Population Ag from Eastern Switzerland is genetically intermediate but resembles more the Austrian populations, a finding that is in accordance with the geographic positions. The heterogeneity of the non-autochthonous populations must be ascribed to additional causes: (1) different geographic origin of the founder individuals, and (2) changes due to founder effects and subsequent genetic drift. Population E is rather similar to the adjacent autochthonous populations B and C. This finding is consistent with the data obtained for the Sod-1 locus (Preleuthner and Pinsker 1993). The close relationship between the re-introduced population E and the autochthonous populations B and C could indicate recent gene flow through migration. However, considering the barrier imposed by the deep cut valley of the rivers Sill and Eisack this explanation appears unlikely. Although the actual origin of population E is not documented, the genetic similarity suggests that the founder individuals were taken from neighbouring autochthonous populations. Population H, although
Fig. 5. Frequencies (in %) of bands obtained by DNA fingerprinting. The eight populations are arranged in west-east direction. Ab, Ag, B, and C are samples from autochthonous populations, the others represent re-introduced populations. Numbers 1-10 represent the ten variable bands.

Genetically more separated, shows also high similarity to the other populations from Western Austria (B, C, E). This result indicates that the founders of population H were most probably taken from these areas. The two re-introduced populations (J and M) from the easternmost part of the Austrian Alps exhibit the strongest differences compared to the other populations from Austria. Population M shows the lowest variability (5 bands) and the common bands 7 and 8, which are present in all Austrian populations, are missing. It seems likely that the variability of this population has been reduced through founder effects and drift. On the other hand, the highest variability was found in population J (10 bands). The band frequencies observed in this population are similar to those in the Swiss population Ag. In addition, the presence of bands 1 and 4, which were found only in the Swiss populations (Ab and Ag) but not in Austria, indicates a possible origin of the founders from the Western Alps.

Allozyme polymorphism and parasite infestation

The gastrointestinal tracts of 162 marmots taken from different geographic locations were examined for endoparasites. Three locations represent autochthonous
Fig. 6. Regional differences in infestation frequencies. The samples were pooled into four geographic groups: A – autochthonous populations (Ab, Ag, B, D), re-introduced-West (E, F, Iv, Zd), re-introduced-Central (G, H, I, J), and re-introduced-East (K, N). A clear differentiation can be observed in the west-east direction.

Populations, the rest is inhabited by re-introduced populations. The locations are arranged along a west-east gradient through the alpine region of Austria. The most common parasite found was the cestode *Ctenotaenia marmotae* (90.1% infested). The intensity of infestation with this parasite proved highly variable (from 1 to 1273 per individual marmot). The nematode *Citellina alpina* is also widespread (62.3% infested) and the intensity of infestation varies between 1 and 1397. Beside these marmot specific parasites there was a number of different nematodes, eg *Ascaris laevis* (10.5%), *Trichuris* spp. (24.1%), and members of the family *Trichostrongylidae* (20.4%). With these species the degree of infestation was less variable (between 1 and 35).

Regional differences in infestation frequencies can be seen in Fig. 6. The samples were pooled into four geographic groups: autochthonous populations (A, B, D), re-introduced-West (E, F, Iv, Zd), re-introduced-Central (G, H, I, J), and re-introduced-East (K, N). A clear differentiation can be observed in the west-east direction. The marmot specific parasites *Ctenotaenia marmotae* and *Citellina alpina* are most abundant in the autochthonous populations. In the re-introduced populations, however, the frequency decreases gradually towards the east. The same is true for *A. laevis* and the genus *Trichuris*. Infestation with *A. laevis* is rather common in the autochthonous populations. Yet, only a single case was detected in the most western location of the re-introduced populations, the others were not infected. With *Trichuris* there is also a slight decrease from the west to the east. An opposite cline is observed for the family *Trichostrongylidae* where the highest frequencies were found in the easternmost populations.

The degree of infestation by endoparasitic worms was investigated in different genotypes of the enzyme loci *Pep-1* and *Sod-1*. The number of parasites was counted for individual marmots and the genotypes were determined electrophoretically. For this investigation the data from all populations had to be pooled.
No significant variation was observed among the Sod-1 genotypes (data not shown). For Pep-1, however, the genotypes differed with respect to the intensity of infestation with the two parasites Citellina alpina and Ctenotaenia marmotae (Fig. 7). With the nematode Citellina alpina the Pep-1 genotype S/S was clearly overrepresented among noninfected individuals. It could be assumed that this genotype provides more resistance against this particular parasite. With the tape worm Ctenotaenia marmotae heterzygotes for the alleles S/F are found at a higher frequency among non-infected or weakly infected (<10 parasites) individuals. This finding indicates a possible advantage of the heterozygotes suggesting that the polymorphism at the Pep-1 locus may be maintained by balancing selection.

**Discussion**

As a result of the distribution history the present range of the species in Austria can be divided into two main areas with different origin of the populations. Autochthonous populations inhabit a continuous range extending over the main ridges of the western parts of the Austrian Alps. In the eastern part, however, non-autochthonous populations, each generated by the release of a few founder individuals in the respective region, occur in a more fragmented area of partly isolated massifs. With respect to the genetic structure environmental factors,
parasites, and human influence have shaped the gene pool of the present populations.

The climatic change after the Pleistocene forced the marmot populations to recede into the present alpine refuge. In the course of this environmental displacement, a severe bottle neck occurred which caused a considerable loss of genetic variation. The reduced variability is still visible at the protein level as revealed by the allozyme studies on recent populations. Only the populations in the western part of the Alps survived to the present day. In these autochthonous populations new variation was generated at the VNTR-loci whereas the variation at structural genes remained low.

Human activities led to the extinction of marmots in the Eastern Alps. The re-introductions of marmots into these areas were carried out with small numbers of individuals. Thus founder effects and drift caused further decrease in variation, eg fixation of alleles. The impact of founder effects and migration barriers on the structure of the disjunct non-autochthonous populations is confirmed by the genetic analysis of both allozymes and VNTR-loci. Whereas the autochthonous populations are characterized by high genetic similarity and common polymorphisms, the non-autochthonous populations exhibit a pattern of patchy variation with no clear geographic trends. Especially the VNTR-patterns show clear divergence of local populations which can be traced back to founder events or different origin of the founder individuals released in the course of re-introduction efforts.

Parasites have probably also contributed as a driving force in the evolution of the marmot populations. The hypothesis, however, that re-introduced populations may be more susceptible to infections by parasites due to their reduced genetic adaptability is not confirmed by the data. In contrast to this expectation, the percentages of infested individuals is even higher in the genetically more variable autochthonous populations. One possible explanation could be that the founder effects in the re-introduced populations have not only reduced the genetic variability but also brought about the loss of parasites in some isolates. In pooled samples, as they were used in our investigation, this phenomenon would show up as a lower percentage of infested individuals. Although all marmot populations studied so far were found to be heavily infested with various parasites of the gastrointestinal tract, these infections do not produce serious symptoms of apparent illness (Jettmar and Anschau 1951, Bergmann and Prosl 1988, Preleuthner 1989, Prosl et al. 1992). Nevertheless, parasites influence the fitness of the host and thus can provide advantages for certain genotypes. An example is given by differences in the degree of infestation among the Pep-1 genotypes. In the case of Ctenotaenia marmotae, balancing selection has probably conserved the variation at the Pep-1 locus. Up to now, reports about different susceptibility of genotypes with respect to microbial or parasitic infections are scarce. Two cases have been described where heterozygosity at several enzyme genes was correlated with lower infection rates (Mulvey et al. 1987, Ferguson and Drahschak 1990). In our case, however, the resistance phenomenon appears to be brought about by a particular
polymorphism at a single locus, or at least by a block of closely linked genes. This aspect certainly deserves more extensive investigations including biochemical analyses of interrelations between peptidase function and molecular defense mechanism against parasite attacks.

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