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Depauperated gene pools in *Marmota m. marmota* are caused by an ancient bottle neck: electrophoretic analysis of wild populations from Austria and Switzerland

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Samples from 15 populations of the Alpine marmot *Marmota m. marmota* (Linnaeus, 1758) were surveyed electrophoretically for allozyme variation. Only 2 out of 50 enzyme loci showed polymorphism. Average heterozygosity was found to be low with 1.2%. No rare alleles were detected among the 8430 genes examined. The geographic variation at the two polymorphic loci (*Pep-1* and *Sod-1*) was analysed in more detail. The distribution pattern of the allele frequencies indicates genetic differentiation between autochthonous and introduced populations. No striking deviations of the genotype distributions from Hardy-Weinberg equilibrium were observed. Thus the population structure is apparently not affected by inbreeding. The obviously diminished genetic variation and the geographic pattern of the allele frequencies at the two variable loci can be best explained by assuming a severe bottleneck in the recent past.

Key words: *Marmota marmota*, allozyme variation, autochthonous versus introduced populations, diminished variation, bottleneck, geographic differentiation

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Introduction

The Alpine marmot *Marmota m. marmota* (Linnaeus, 1758) is continuously distributed over the entire mountain range of the Alps. In addition, dispersed patches of isolated populations are found in other mountainous regions of Europe, e.g. in the Tatra, the Pyrenées, and the Apennine Mountains (Krapp 1978, Sala *et al.* 1992). The present distribution is partly the result of human activities. Only the populations from the western region of the Alps are autochthonous. Large areas, however, are now inhabited by marmot populations which have been repeatedly introduced by local hunters over the rather short period of about 150 years. For these regions of the Eastern Alps no unequivocal reports prove the occurrence of marmots before the first documented introduction in 1860 (Zimmeter 1886, Niethammer 1963). This means that marmot populations have either failed

to recolonize this area after the last glaciation, or have become extinct by human hunters in prehistoric times. The question is still under investigation (M. Preleuthner, in prep.) and no final answer can be given on the basis of our present knowledge.

The artificial (re)colonization was mainly conducted by releasing small groups of up to six founder individuals into the new habitat. This colonization strategy may have resulted in a massive impact on the genetic structure of the populations by reducing the amount of variation, as it has been documented for cloven-hooved game species (Hartl 1986, Hartl *et al.* 1986, Hartl 1990, Randi *et al.* 1990). Furthermore, the subdivision of the populations into family groups as well as the mating system within these groups (Arnold 1990a) could give rise to the fixation of alleles by genetic drift and to inbreeding. Because of the historic events and the species specific social substructure modeling the genetic make up of the populations, marmots appear to be predisposed for the loss of genetic variety. The electrophoretic analysis of a sample from Berchtesgaden (Arnold 1990a) indicates low variability in this local population. Therefore it was the major aim of the present study to explore the marmot's genetic status in more detail and on a larger geographic scale. The results are thought to provide valuable insights which could be of practical importance for the management of the gene pool, especially in combination with efforts to preserve the gene resources of the species.

Gel electrophoresis was employed in order to estimate the amount of genetic variation at the protein level both within and between populations. Up to now, genetic investigations on marmots are scarce. Among the 14 marmot species described so far (Hoffmann *et al.* 1979, Barash 1989), allozyme analyses have been carried out on the yellow-bellied marmot *M. flaviventris* (Schwartz and Armitage 1980, 1981) and on the woodchuck *M. monax* (Wright *et al.* 1987). These studies revealed rather high levels of polymorphism. In *M. flaviventris* 40% of the loci were polymorphic and average heterozygosity measured 7.5%. The corresponding values in *M. monax* were 25% and 5.3% respectively. Thus both species have heterozygosity values well above the average (H = 3.6%) obtained for the mammalian class (Nevo 1978). Close relatives of the genus *Marmota* surveyed by allozyme techniques are the black-tailed prairie dog *Cynomys ludovicianus* (Chesser 1983) and Belding's ground squirrel *Spermophilus beldingi* (Hanken and Sherman 1981) with heterozygosity values of 6.6% and 10.7% respectively. These data indicate that allozyme variation in the sciurid family is comparably high.

The apparent lack of genetic data on *M. marmota* stands in sharp contrast to the wealth of information on other aspects of marmot biology. Over the last decades Alpine marmots have been thoroughly investigated with respect to their geographic distribution (e.g. Forter 1975, Chiesura 1992, Neet 1992, Ramousse *et al.* 1992), sociobiology and ecology (e.g. Psenner 1960, Lattmann 1973, Barash 1976, 1989, Arnold 1990a, b), physiology (e.g. Arnold 1988, Türk and Arnold 1988) and parasitology (e.g. Prosl *et al.* 1992). In addition to the mere scientific interest, Alpine marmots are also of economic significance. In former times they have been

intensively exploited for food, fur, and pharmaceutical purposes. Nowadays they serve mainly as objects of hunting and as an attraction for tourism in the high regions of the Alps.

In order to determine the most important population genetic parameters of Alpine marmots, samples were taken from different regions of the Alps. The areas chosen for the study comprise both autochthonous and introduced populations. Among the autochthonous populations three samples were taken from the western part of Austria and one sample from Switzerland (two populations pooled). Additional data from a previous study by Arnold (1990a) were included for comparison. The ten introduced populations are from the central and eastern parts of Austria, including one sample from the eastern margin of the continuous distribution area. Thus the samples cover the entire geographic range inhabited by marmots in Austria (Fig. 1).

Material and methods

Populations and sampling procedures

Population samples were collected from different geographic regions (Fig. 1): 13 from Austria (Eastern Alps) and 2 from Switzerland (Western Alps). In Table 1 the sample areas are characterized with respect to geology and altitude. In addition, it is indicated whether the populations are autochthonous or introduced. In the latter case, the origin of the introduced populations, as far as it is known, is also given.

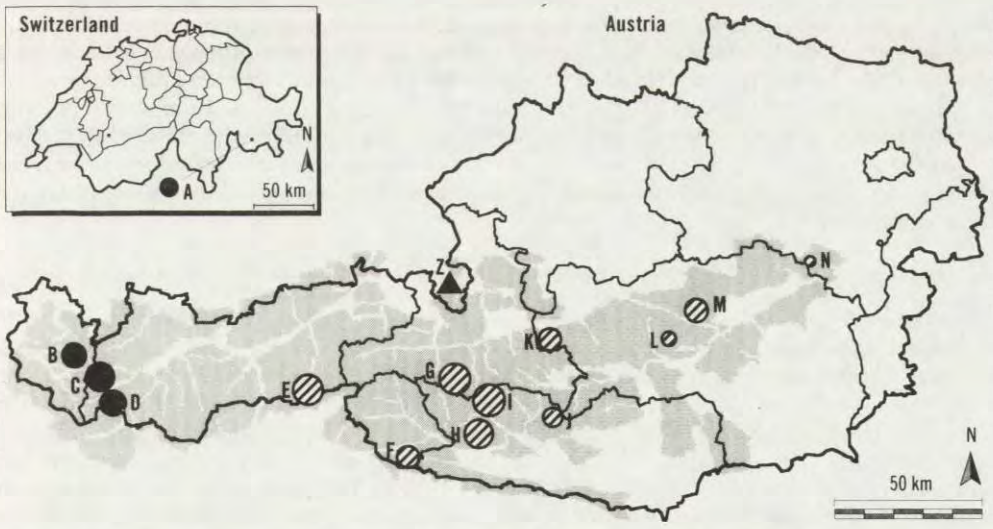

Fig. 1. Geographic location of the sample areas of the present study (dots) and the sample from Berchtesgaden, Germany (black triangle; Arnold 1990a). Dot size is proportional to sample size. Shaded areas show the mountains inhabited by *M. marmota* and adjacent areas. The map of Switzerland gives the location of the two populations pooled as area "A". All area codes are listed in Table 1. Autochthonous populations are given as black symbols, introduced are hatched.

Table 1. Characterization of sampling areas. The areas are listed from west to east. The altitude is given for the site of shooting. The presumptive provenance is shown for the introduced populations. a – autochthonous, i – introduced, ? – unknown provenance.

Code	Mountain region	Geology	Meters above sea-level	a/i	Provenance of introduced populations
A	Western Alps	–	1570 – 2000	a	
B	Lechquellengebirge	limestone	1600 – 2400	a	
C	Verwallgruppe	limestone	1600 – 2600	a	
D	Samnaungruppe	slate, granite and gneiss	1700 – 2700	a	
E	Zillertaler Hauptkamm	granite and gneiss	1100 – 2500	i	?
F	Lesachtalkette	greywacke	1350 – 2600	i	Styria and Carinthia
G	Glocknergruppe	granite and slate	1500 – 2600	i	?
H	Kreuzeckgruppe	crystalline slate	1300 – 2500	i	Tyrol
I	Ankogelgruppe	granite and gneiss	1600 – 2300	i	?
J	Turracher Nockberge	crystalline and slate	1750 – 2200	i	Nockberge
K	Schladminger Tauern	granite and gneiss	1000 – 2100	i	Tyrol, Lechtaler Alpen
L	Seckauer Tauern	granite, gneiss and crystalline slate	1800 – 2000	i	?
M	Eisenerzer Alpen	greywacke	900 – 1650	i	Tyrol
N	Rax	limestone	1650 – 1850	i	?

In order to get the permission for organizing the sampling by local hunters, a good cooperation with the hunting authorities had to be established. Sampling was done during the annual hunting season in August and September. As the quantity of the obtained material directly depends on hunting activity per year and region, the gathering of all specimens took several successive years (1984 – 1988). It should be emphasized that the material for this investigation was obtained as a byproduct of the regular hunting. No marmots were killed for purposes of the scientific study.

The samples were taken from 593 freshly killed Alpine marmots. Tissue samples of liver, kidney, heart and muscle were separately packed into plastic bags. Within a few hours (maximally 2 days) the samples were stored at –25°C. Since the enzymes start to degrade shortly after the shooting, the time lag between sampling and storage, as well as the weather conditions, were decisive for the quality of the samples.

Sample preparation and electrophoresis

Enzyme electrophoresis was carried out in horizontal starch gels according to Ayala *et al.* (1972). Prior to electrophoresis, tissue extracts were prepared from small pieces of about 2 cm^3. In preliminary tests the best results were obtained with liver extracts which were subsequently used in the main investigation. The equal volume of extraction buffer (0.01 M phosphate buffer pH 7.0 containing 0.01 mM $MgCl_2$) was added to the extracts. Homogenization was done in a kinematic high-frequency dispersing system (Ultraturax). Cooling was applied throughout homogenization. Afterwards the dispersions were centrifuged (5000 rpm) for 40 min at 4 – 5°C. The supernatant was absorbed onto filter wicks (Whatman 3MM, 4 × 11 mm) which were inserted into a cathodally positioned slice across the gel. The wicks were removed after 20 min. Depending on the buffer system the gels were run for 5 – 17 hours.

The 30 enzyme systems and the codes for separation and staining methods are listed in Table 2. Five buffer systems (designated A–E) were employed: (A) Tris / citric acid pH 8.0 (Manlove *et al.* 1975), (B) Phosphate pH 7.4 (Csaikl *et al.* 1980), (C) Tris / malat pH 7.7 (Selander *et al.* 1971),

Table 2. List of enzyme systems surveyed. Abbreviations are according to Harris and Hopkinson (1976), and Hillis and Moritz (1990) based on IUBNC (1984). *n* – number of genes examined in the present study. Buffer systems (A–E) and references for staining procedures (a–h) are listed in the text.

Enzyme	Abbr.	E.C. number	Buffer system	Enzyme staining	Loci	*n*
1	2	3	4	5	6	7
Acid phosphatase	ACP	E.C. 3.1.3.2	A	c	*Acp-1*	76
					Acp-2	76
Aconitase hydratase	ACOH	E.C. 4.2.1.3	C	b	*Acoh-1*	102
					Acoh-2	102
Adenosine deaminase	ADA	E.C. 3.5.4.4	A	a	*Ada-1*	104
Adenylate kinase	AK	E.C. 2.7.4.3	A	a	*Ak-1*	114
					Ak-2	114
Alcohol dehydrogenase	ADH	E.C. 1.1.1.1	B	b	*Adh-1*	216
					Adh-2	216
					Adh-3	216
Aldolase	ALD	E.C. 4.1.2.13	D	b	*Ald-1*	78
					Ald-2	78
					Ald-3	78
Alkaline phosphatase	ALP	E.C. 3.1.3.1	E	b	*Alp-1*	50
Aminoacylase	ACY	E.C. 3.5.1.14	A	d	*Acy-1*	76
Aspartate aminotransferase	AAT	E.C. 2.6.1.1	A	a	*Aat-1*	156
					Aat-2	156
Catalse	CAT	E.C. 1.11.1.6	A	c	*Cat-1*	100
Creatine kinase	CK	E.C. 2.7.3.2	A	b	*Ck-1*	74
					Ck-2	74
Diaphorase	DIA	E.C. 1.6.-.-	A	b	*Dia-1*	50
Esterase (nonspecific)	EST	E.C. 3.1.1.-	B	b	*Est-1*	78
					Est-2	78
					Est-3	78
Fumarate hydratase	FUMH	E.C. 4.2.1.2	A	a	*Fumh-1*	58
Glucose dehydrogenase	GCDH	E.C. 1.1.1.47	A	b	*Gcdh-1*	154
Glucose-6-phosphate dehydrogenase	G6PDH	E.C. 1.1.1.49	A	f	*G6pdh-1*	152
Glucose-6-phospate isomerase	GPI	E.C. 5.3.1.9	A	g	*Gpi-1*	258
Glutamate dehydrogenase	GTDH	E.C. 1.4.1.2	A	c	*Gtdh-1*	154
Glycerol-3-phosphate dehydrogenase	G3PDH	E.C. 1.1.1.8	C	c	*G3pdh-1*	156
					G3pdh-2	156
Hexokinase	HK	E.C. 2.7.1.1	A	c	*Hk-1*	122
					Hk-2	122
Isocitrate dehydrogenase	IDH	E.C. 1.1.1.42	A	c	*Idh-1*	194
					Idh-2	68
L-Lactate dehydrogenase	LDH	E.C. 1.1.1.27	B	c	*Ldh-1*	180
					Ldh-2	180
Malate dehydrogenase	MDH	E.C. 1.1.1.37	A	f	*Mdh-1*	146
					Mdh-2	146
Malate dehydrogenase	MDHP	E.C. 1.1.1.40	A	h	*Mdhp-1*	200
Mannose-6-phosphate isomerase	MPI	E.C. 5.3.1.8	B	e	*Mpi-1*	174
Peptidases						
Peptidase (L-leucyl-L-alanine)	PEP-A	E.C. 3.4.-.-	A	a	*Pep-1*	1040
Peptidase (L-leucylglycylglycine)	PEP-B				*Pep-2*	120
Peptidase (L-phenylalanine-L-proline)	PEP-D				*Pep-3*	120
Cyotosol aminopeptidase	PEP-E	E.C. 3.4.11.1	B	b	*Pep-4*	478

Table 2 – concluded.

1	2	3	4	5	6	7
Phosphoglucomutase	PGM	E.C. 5.4.2.2	A C	b	*Pgm-1* *Pgm-2*	60 240
Phosphogluconate dehydrogenase	PGDH	E.C. 1.1.1.44	B	g	*Pgdh-1*	200
Sorbitole dehydrogenase	SORDH	E.C. 1.1.1.14	C	c	*Sordh-1*	198
Superoxide dismutase	SOD	E.C. 1.15.1.1	B	b	*Sod-1*	814
Total					50	8430

(D) Tris / maleic anhydride pH 7.4 (Harris and Hopkinson 1976), and (E) Boric acid / NaOH pH 8.1 (Ayala *et al.* 1972). Procedures for enzyme staining (designated a–h) were taken from the following authors: (a) Allendorf *et al.* (1977), (b) Harris and Hopkinson (1976), (c) Shaw and Prasad (1970), (d) Quavi and Kit (1980), (e) Nichols *et al.* (1973), (f) Brewer (1970), (g) Manlove *et al.* (1976), and (h) Ayala *et al.* (1972). The genetic interpretation of electrophoretic patterns was based on the principles outlined by Harris and Hopkinson (1976), Richardson *et al.* (1986), and Hillis and Moritz (1990). Fifty genetically independent loci could be distinguished.

Results

Genetic variation

A total number of 30 marmot enzyme systems was screened for genetic variation (Table 2). According to the most likely genetic interpretation, the proteins of 15 enzyme systems appear to be coded by single genes. From the comparison with data from other studies on mammalian enzyme systems (Harris and Hopkinson 1976, Richardson *et al.* 1986, Hillis and Moritz 1990), different activity zones in the zymograms were recognized as genetically independent isozymes in the other 15 enzyme systems. Thus alltogether 50 presumptive gene loci were identified with sufficient resolution and used for the further survey.

Studying samples derived from different populations (a total of 50 – 478 genes per locus, average 137), 48 enzyme genes were found to be completely monomorphic. For these 48 loci a total number of 6576 genes was analysed, but no rare variants could be detected. Since these genes were considered useless for questions of geographic differentiation and population structure, they were not further examined in the rest of the material. Two enzyme loci, however, showed pronounced polymorphism: *Pep-1* and *Sod-1*. For the isozyme PEP-1 leucyl-alanine was used as substrate and the 3-banded pattern in the heterozygotes indicates a dimeric structure of the protein. Therefore it seems to correspond to the human isozyme PEP-A described by Harris and Hopkinson (1976). The other peptidases had different substrate specifities (Table 2) and proved to be monomorphic in the samples studied. For SOD only one isozyme (SOD-1) was detected. As with PEP-1, the heterozygotes showed a 3-banded zymogram characteristic for dimeric enzymes.

Allele frequencies and geographic variation

The polymorphic loci *Pep-1* and *Sod-1* were investigated in more detail. As many samples as possible were tested from the available material (520 individuals for *Pep-1*, 407 for *Sod-1*). Two electrophoretic alleles could be distinguished at each of the two loci, but no additional rare variants were found in spite of the large total sample size. The allele frequencies calculated from the observed genotype distributions in the various populations are given in Table 3. Additional data from the Berchtesgaden area (Arnold 1990a) are included for comparison. The *Pep-1* locus proved to be polymorphic in all provenances. The frequencies of the fast allele range from 14.4% (population B) to 87.5% (population J). Pooling the data of all populations (A–N), the slow and the fast allele are found in roughly equal proportion (54.1% : 45.9%). With *Sod-1* the fast allele appears predominant in all populations except for the areas D and E. High frequency of the fast allele seems to be characteristic for the ten non-autochthonous populations. Four of these population samples (I, J, L, N) were even monomorphic for this allele.

The distribution pattern of the allele frequencies at the two polymorphic loci indicates considerable genetic heterogeneity of the total sample. A χ^2-test for heterogeneity reveals significant differences between populations for both polymorphic genes (*Pep-1*: $\chi^2 = 392.5$, df = 14, $p < 0.001$; *Sod-1*: $\chi^2 = 273.3$, df = 14, $p < 0.001$). This result suggests that the marmot populations do not belong to one large panmictic assembly but instead are partially isolated. Differences between particular populations have been tested in pairwise comparisons. The results of the statistic analysis (χ^2-test) are given in Tables 4 and 5. For both enzymes, statistically significant differences in allele frequencies were observed in about 60% of all tests. In *Pep-1* 50% of the pairwise comparisons proved highly significant ($p < 0.001$), in *Sod-1* the respective value was 39%. This finding supports the assumption of considerable divergence between the populations. A higher proportion of significant differences is obtained in comparisons between autochthonous *versus* introduced populations indicating differences in allele frequencies between these two types of populations. In a χ^2-test carried out with pooled data separating autochthonous and introduced populations, the differences proved highly significant for both enzymes (*Pep-1*: $\chi^2 = 192.1$; *Sod*-1: $\chi^2 = 149.3$; df = 1, $p < 0.001$ for both enzymes).

Clustering into homogenous groups

The geographic distribution of allele frequencies is presented in Fig. 2 (*Pep-1*) and Fig. 3 (*Sod-1*). With both enzymes the variation between populations is not erratic. The observed pattern is in accordance with geographic affinities and may also reflect the presumed historic origin of the populations. χ^2-tests were used to prove homogeneity of the apparent clusters. In *Pep-1* (Fig. 2) four of the five autochthonous populations A, B, C, and Z form a homogenous cluster. Only population D is clearly distinct from this group. The introduced populations are more

Table 3. Allele frequencies (in %, s – slow, f – fast), observed (H_o) and expected (H_e) heterozygosity (in %), and inbreeding coefficients (F_{IS}) at 2 enzyme loci analysed in different populations of *M. marmota*. n – number of genes examined, p – deviation from Hardy-Weinberg equilibrium (level of significance: * – 0.05, ** – 0.01, *** – 0.001; df = 1). Area codes A to N are listed in Table 1. The areas are presented left to right according to the geographic location in the west-east direction. A–N: data from populations of the present study. Z – additional data obtained by Arnold (1990a) for the Berchtesgaden area for comparison.

Enzyme		Autochthonous populations					Introduced populations										
Areas		A	B	C	D	Z	E	F	G	H	I	J	K	L	M	N	A-N
PEP-1	s	68.5	85.6	79.3	29.7	84.8	58.2	64.1	55.7	44.4	22.5	12.5	31.7	50.0	71.4	83.3	54.1
	f	31.5	14.4	20.7	70.3	15.2	41.8	35.9	44.3	55.6	77.5	87.5	68.3	50.0	28.6	16.7	45.9
	n	54	90	116	74	644	110	64	106	90	120	48	60	26	70	12	1040
	H_o	48.1	24.4	24.1	43.2	28.0	29.1	46.9	43.4	44.4	35.0	16.7	43.3	69.2	28.6	33.3	$\bar{H}_o$ 37.9
	H_e	43.1	24.7	32.8	41.8	25.8	48.7	46.0	49.4	49.4	34.9	21.9	43.3	50.0	40.8	27.8	$\bar{H}_e$ 39.6
	p			*			**										
	F_{IS}	−0.12	0.01	0.26	−0.03	−0.08	0.40	−0.02	0.12	0.10	0.00	0.24	0.00	−0.38	0.30	−0.20	$\bar{F}_{IS}$ 0.05
SOD-1	s	30.8	19.7	34.3	45.5	47.4	57.3	8.0	16.3	7.1	0.0	0.0	5.6	0.0	6.1	0.0	16.5
	f	69.2	80.3	65.7	54.5	52.6	42.7	92.0	83.7	92.9	100.0	100.0	94.4	100.0	93.9	100.0	83.5
	n	52	66	70	44	321	82	50	80	56	118	46	54	24	66	6	814
	H_o	38.5	15.2	45.7	36.4	52.3	56.1	8.0	12.5	14.3	0.0	0.0	11.1	0.0	6.1	0.0	$\bar{H}_o$ 17.4
	H_e	42.6	31.6	45.1	49.6	49.9	48.9	14.7	27.2	13.3	0.0	0.0	10.5	0.0	11.4	0.0	$\bar{H}_e$ 21.1
	p		***						***								
	F_{IS}	0.10	0.52	−0.01	0.27	−0.05	−0.15	0.46	0.54	−0.08	----	----	−0.06	----	0.47	----	$\bar{F}_{IS}$ 0.21

Table 4. Differences in allele frequencies at the *Pep-1* locus between the various population samples (A–N, Z). χ^2-values obtained in pairwise comparisons are given below the diagonal. Levels of significance (* – 0.05, ** – 0.01, *** – 0.001, df = 1) are shown above the diagonal. The comparisons autochthonous vs autochthonous, autochthonous vs introduced, and introduced vs introduced are separated by lines.

	Autochthonous populations					Introduced populations									
	A	B	C	D	Z	E	F	G	H	I	J	K	L	M	N
A		*		***	**				**	***	***	***			
B	5.9			***		***	**	***	***	***	***	***	***	*	
C	2.3	1.3		***		***	*	***	***	***	***	***	**		
D	18.9	52.9	46.3		***	***	***	***			*			***	***
Z	9.6	0.0	2.2	121.7		***	***	***	***	***	***	***	***	**	
E	1.6	17.8	11.8	14.4	43.0					***	***	***			
F	0.3	9.6	5.0	16.3	17.6	0.6			*	***	***	***			
G	2.5	20.5	14.2	11.8	49.5	0.1	1.2			***	***	**		*	
H	7.9	33.4	26.8	3.7	79.8	3.7	5.8	2.4		***	***			***	*
I	33.9	81.8	76.2	1.3	209.3	30.6	30.9	26.3	11.4			*	**	***	***
J	32.7	69.7	63.0	4.9	144.6	28.3	29.9	25.2	14.4	2.2			***	***	***
K	15.4	45.4	38.5	0.1	97.7	10.9	13.0	8.9	2.5	1.8	5.5			***	***
L	2.6	14.7	9.5	3.5	21.9	0.6	1.5	0.3	0.3	8.1	12.4	2.6		*	
M	0.1	4.8	1.5	25.0	8.2	3.2	0.8	4.4	11.7	43.9	39.7	20.5	3.9		
N	1.1	0.0	0.1	12.7	0.0	2.9	1.7	3.4	6.4	20.0	24.6	11.1	3.8	0.7	

Table 5. Differences in allele frequencies at the *Sod-1* locus between the various population samples (A–N, Z). χ^2-values obtained in pairwise comparisons are given below the diagonal. Levels of significance (* – 0.05, ** – 0.01, *** – 0.001, df = 1, nd – not determined: no χ^2 – test possible, expected value < 1) are shown above the diagonal. The comparisons autochthonous vs autochthonous, autochthonous vs introduced, and introduced vs introduced are separated by lines.

	Autochthonous populations					Introduced populations									
	A	B	C	D	Z	E	F	G	H	I	J	K	L	M	N
A					*	**	**	*	**	***	***	***	**	***	
B	1.9			**	***	***			*	***	**	*	*	*	
C	0.2	3.7			*	**	***	*	***	***	***	***	***	***	
D	2.2	8.3	1.4				***	***	***	***	***	***	***	***	*
Z	5.3	18.5	4.3	0.1			***	***	***	***	***	***	***	***	*
E	9.0	21.5	8.0	1.6	2.9		***	***	***	***	***	***	***	***	**
F	8.4	3.1	11.3	17.3	29.1	31.9				**					
G	3.9	0.3	6.5	12.4	27.9	29.3	1.8			***	**		*		
H	10.0	4.0	13.3	19.8	33.8	36.0	0.0	2.5		**					
I	40.1	25.0	46.4	61.2	93.1	88.4	9.7	20.5	8.6		nd	**	nd	**	nd
J	16.9	10.3	19.9	26.9	39.0	41.7	3.8	8.3	3.4	nd			nd		nd
K	11.4	5.1	14.8	21.5	35.3	37.5	0.2	3.5	0.1	6.7	2.6				
L	9.4	5.5	11.0	15.5	20.9	24.7	2.0	4.5	1.8	nd	nd	1.4			nd
M	12.6	5.5	16.6	24.0	41.5	42.5	0.2	3.6	0.1	7.3	2.9	0.0	1.5		
N	2.5	1.4	3.0	4.5	5.4	7.4	0.5	1.1	0.5	nd	nd	0.4	nd	0.4	

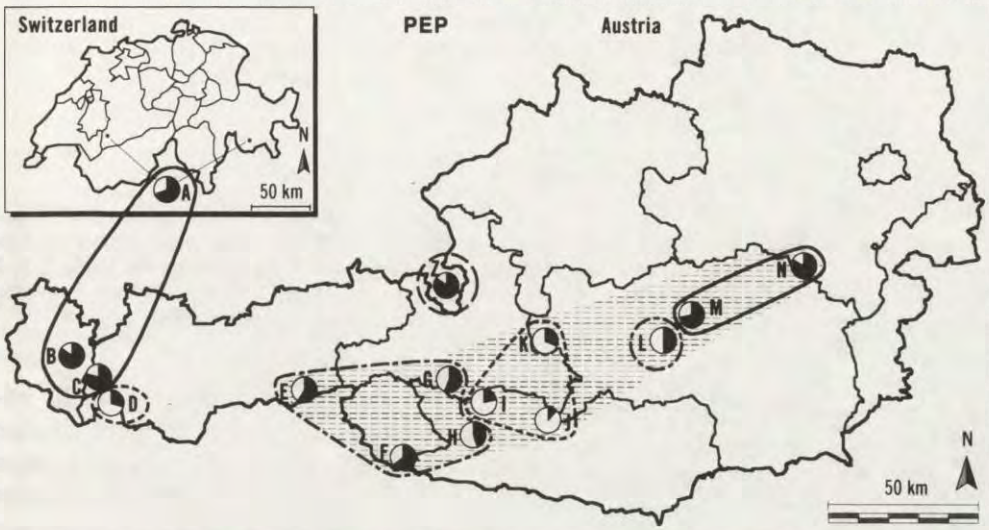

Fig. 2. Piecharts showing the percentage of slow (black) and fast (white) alleles of *Pep-1* in sample areas from Austria (13), Switzerland (2 samples pooled) and Berchtesgaden (1). Area codes are listed in Table 1. Introduced populations are indicated by dashed background. Variation of allelic frequencies is higher among non-autochthonous populations.

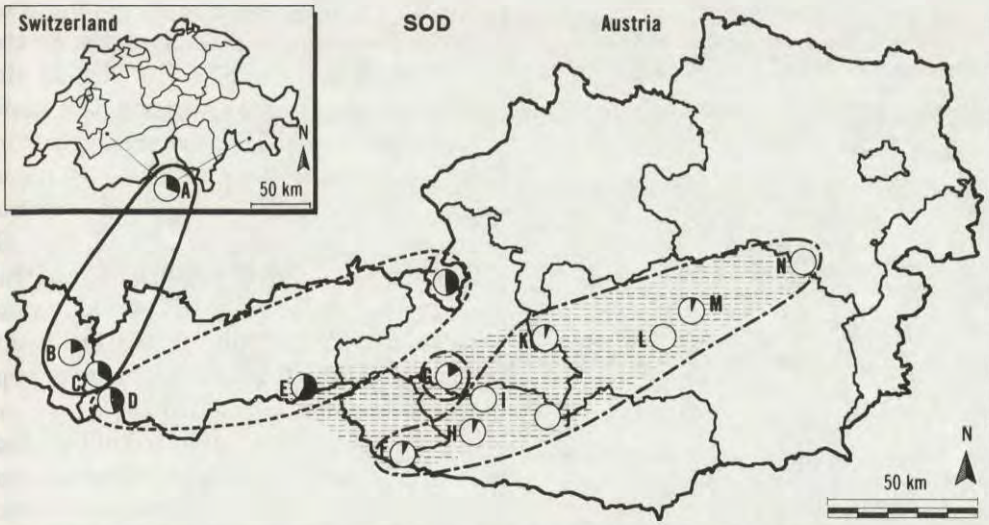

Fig. 3. Piecharts showing the percentage of slow (black) and fast (white) alleles of *Sod-1* in sample areas from Austria (13), Switzerland (2 samples pooled) and Berchtesgaden (1). Area codes are listed in Table 1. Introduced populations are indicated by dashed background. Variation of allelic frequencies is higher among non-autochthonous populations. Four population samples (I, J, L, N) were found monomorphic for the fast allele.

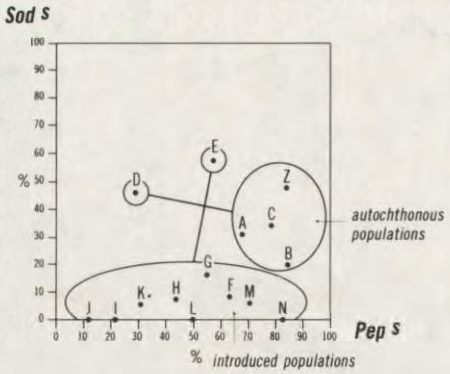

Fig. 4. Frequencies (in %) of the alleles *Sod-1*^{s} and *Pep-1*^{s} in different *M. marmota* populations from Austria and Switzerland. For area codes see Table 1: A–N – present investigation, Z – sample from Berchtesgaden (Arnold 1990a) for comparison.

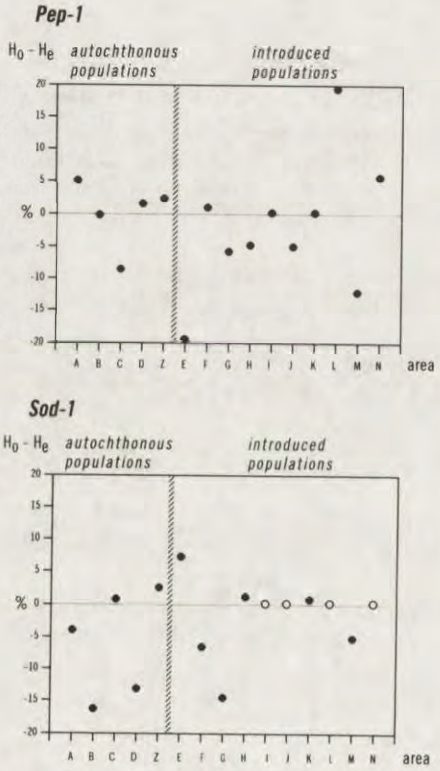

Fig. 5. Deviations from expected heterozygosity at the two polymorphic loci *Pep-1* and *Sod-1* in populations A–N and Z. No substantial differences can be detected between autochthonous and introduced populations. $H_o - H_e$ = difference between observed (H_o) and expected (H_e) heterozygosity. ● – polymorphic populations, ○ – monomorphic populations (no heterozygotes).

heterogenous. However, geographically adjacent populations tend to be more similar in their allele frequencies. Three subgroups (E+F+G+H+L, K+I+J, M+N) can be distinguished. Subgroup K+I+J shows similarities to the autochthonous population D, subgroup M+N resembles cluster A+B+C of the autochthonous group.

A similar grouping of the populations is derived from allele frequencies at the *Sod-1* locus. As in *Pep-1*, a cluster is formed by the autochthonous populations A+B+C. Populations D+Z cluster with the introduced population E. The rest of the introduced populations appears as a more or less homogenous group characterized by low frequencies of the slow allele. In populations I+J+L+N the slow allele was not observed in the samples. Population G, which is located at the western edge of this area, proved different in an overall homogeneity test. With this particular population, gene flow from autochthonous populations cannot be excluded.

Considering the information of both loci the difference between autochthonous and introduced populations becomes obvious. The relation between the frequencies of the respective slow variants of *Pep-1* and *Sod-1* is depicted in Fig. 4. In the diagram the autochthonous and the introduced

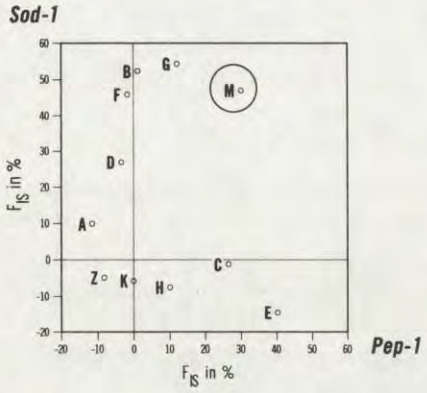

Fig. 6. Correlation between inbreeding coefficients (F_{IS}) at the *Pep-1* and *Sod-1* locus. With the exception of population M there seems to be no indication for nonrandom mating within populations.

populations fall into two separated clusters. The autochthonous populations cluster more densly. The higher variation of the introduced populations is mainly caused by the *Pep-1* locus. Populations D and E are distinct from the rest and thus do not fit into this general pattern.

Genotypic variation within and between populations

Observed (H_o) and expected (H_e) heterozygosity values at the loci *Pep-1* and *Sod-1* are compared in Table 3. Significant deviations are observed for populations C ($p < 0.05$) and E ($p < 0.01$) at the *Pep-1* locus, and for populations B and G at the *Sod-1* locus ($p < 0.001$ for both populations). The graphic presentation of the deviations from expected heterozygosities is shown in Fig. 5. At both polymorphic loci the number of positive and negative deviations is about the same. In general, the distribution of the genotypes appears to be in accordance with the Hardy--Weinberg equilibrium.

Genetic differentiation

The genetic differentiation of marmots among and within the populations was analyzed by using Wright's (1965) *F*-statistics as modified by Nei (1977). Inbreeding coefficients (F_{IS}) for all populations are given in Table 3. There are about as many negative as positive F_{IS} values within the populations. The average inbreeding coefficients are quite low: $F_{IS} = 0.05$ (*Pep-1*) and $F_{IS} = 0.21$ (*Sod-1*; monomorphic populations I, J, L, N not included). The F_{IS} values obtained for the two loci are plotted in Fig. 6. Obviously the deviations from expected heterozygosity at the *Pep-1* and *Sod-1* loci are not correlated. No prominent differences in F_{IS} values between autochthonous (*Pep-1*: 0.01, *Sod-1*: 0.16) and introduced (*Pep-1*: 0.05, *Sod-1*: 0.20) populations could be detected. These findings indicate that in general inbreeding does not play a substantial role for the population structure of Alpine marmots. The only exception is population M where high F_{IS} values are found for both genes. The average fixation index (F_{ST}) and the overall inbreeding coefficient (F_{IT}) were also determined: $F_{ST} = 0.20$ and $F_{IT} = 0.24$ for *Pep-1*,

F_{ST} = 0.23 and F_{IT} = 0.37 for *Sod-1*. These values suggest that the major fraction of the total inbreeding coefficient is due to genetic drift in separated populations rather than to nonrandom mating within populations.

Linkage disequilibrium

Nonrandom association between the genotypes of the two loci was tested in three groups with sufficient sample size: the total sample (populations A–N), and two single populations (A and G). The observed frequencies of the two-locus--genotypes were in good accordance with the expected values calculated from the allele frequencies at the two loci ($0.0 \leq \chi^2 \leq 0.2$, df = 4, ns). Thus the data from the present material do not indicate linkage disequilibrium between alleles of the two different genes.

Discussion

Reduced variability

The most striking result emerging from this investigation is the substantially reduced level of genetic variation in *M. marmota*. Only 2 out of 50 loci (4%) were found polymorphic and average heterozygosity measured 1.2% (all samples pooled). Although a rather large sample of 6576 genes has been screened for the 48 monomorphic loci, no rare variants (with frequencies < 1%) were detected. Even with the 2 variable genes *Pep-1* and *Sod-1* no additional alleles were found among the 1854 genes examined. This is consistent with a previous study conducted by Arnold (1990a) on a local population sample from Berchtesgaden. Thus the obvious lack of variation is not limited to a restricted area but appears as a general phenomenon of all populations studied so far. Comparing these findings with those obtained for the related species *M. flaviventris* and *M. monax* which exhibit normal levels of genetic variety (Schwartz and Armitage 1980, 1981, Wright *et al.* 1987), the gene pool of *M. marmota* seems to be deprived. Since studies on other rodents from related genera revealed a degree of polymorphism comparable to that of *M. flaviventris* (Hanken and Sherman 1981, Chesser 1983, Nadler *et al.* 1985, Daley 1992), it has to be assumed that the ancestor of *M. marmota* possessed a variable gene pool. However, this variation has somehow been lost in the recent evolutionary history of the lineage. Several explanations could be invoked: (1) inbreeding within populations, (2) founder effects and successive genetic drift in separated subpopulations, (3) selective adaptation of the gene pool to a particular ecological niche, and (4) a severe bottleneck in the recent past.

These possibilities can be discussed on the basis of our results, taking into account the allelic variation of the loci as well as the geographic distribution pattern of the remaining polymorphism. The first explanation, inbreeding within local groups, appears unlikely. According to observations of sociobiologists (Schwartz and Armitage 1980, Arnold 1990a) the social substructure of marmot

species (*M. flaviventris*, *M. marmota*) does not promote incestuous matings between closely related individuals. The population genetic data also do not provide evidence for considerable inbreeding. The deviations from Hardy-Weinberg expectations in the 15 population samples of *M. marmota* were more or less negligible. Moreover, though local inbreeding would result in genetic uniformity of subpopulations as a consequence of the stochastic loss of alleles, different allelic variants of the original polymorphism should have become fixed in the various populations. This, however, is definitely not the case. The second explanation, assuming genetic drift within populations as the major cause, can be refuted with the same argument. Furthermore, the maintenance of polymorphism at the two variable loci in most populations is not consistent with the hypothesis postulating strong drift effects, e.g. through permanently occurring fluctuations in population size. The third explanation, which supposes particular selective forces driving the gene pool to an adaptive optimum through the fixation of the best allele at each locus, is also considered inadequate. It is hard to imagine that *M. flaviventris*, a closely related species which resides in a similar habitat and has a comparable social structure, should be less adapted than *M. marmota*. But even under extreme selective pressure, rare alleles are expected to arise constantly from random mutations and should be detectable in a large population sample. The absence of rare alleles is considered as a classic evidence for founder effect (Halliburton and Barker 1993) and thus confirms the fourth explanation, a historical drop in the total population size of the species down to a few individuals. A longer lasting bottleneck or repeatedly occurring severe decreases of the population number would radically remove the genetic variation from the gene pool. The restoration of the original variation levels by the natural mutation rate, however, is a very slow process. It can be speculated that the presumed bottle neck took place at the end of the last glaciation, when the area inhabited by marmot populations was drastically reduced by an altitudinal shift of the timber line. As a consequence of the climatic change the once widely distributed marmots had to retreat to an ecological refuge provided by the higher regions of the Alps. Although this hypothesis sounds plausible, bottlenecks can as well be ascribed to other environmental factors (e.g. viral or bacterial plagues, parasites, extinction by early human hunters, destruction of the habitat).

Accepting the bottleneck hypothesis, the still existing polymorphism at the two variable loci *Pep-1* and *Sod-1* remains to be explained. Both enzymes possess a dimeric quaternary structure and thus belong to a generally less variable class of proteins (Ward 1977). Nevertheless, the two loci are polymorphic and have retained their variation almost throughout the distribution range. *Pep-1* is polymorphic even in the peripheral isolate represented by the small population sample N from the eastern margin of the continuous distribution area. It is tempting to postulate an adaptive value for these polymorphisms which have been constantly maintained in spite of a species wide bottleneck and subsequent genetic drift effects in local populations. Balancing selection may be a possible cause. Although

no excess of heterozygotes was observed in the adult population, this selection mechanism cannot be excluded since it could also act on late fitness components like fertility. In this case no deviation from the expected genotype distributions can be expected. Moreover, other selective mechanisms (e.g. frequency dependent selection) are also possible as a balancing force. A coordinated maintenance of variation at both loci by genetic hitchhiking (Hedrick 1982) cannot be inferred from the data. The distribution of the two-locus-genotypes does not indicate chromosomal linkage.

Geographic differentiation

Because of the reduced level of genetic variability, any inferences on geographic differentiation appear questionable. Nevertheless, the two variable loci display some remarkable geographic patterns that may reflect the history of the colonization. As can be seen in Fig. 4, autochthonous and introduced populations are genetically distinct. Moreover, the autochthonous populations seem to be more homogenous in their genetic composition. This finding suggests that the colonization process has generated genetic changes. Independent release of a small number of individuals at various sites may have shifted the initial allele frequencies of the introduced populations. Subsequent genetic drift favoured by the patchiness of suitable marmot habitats may have inforced this random differentiation of the founder populations giving rise to the present heterogeneity. Similarities between adjacent populations, however, could be interpreted as an indication for gene flow at a limited geographic scale (Figs 2 and 3). Another explanation for heterogeneity among the introduced populations are differences already present in the source populations. In some cases individuals from different populations were released in the same area. Population F, which was founded by individuals from three areas (G, H, and M), can be seen as an example of such a mixed population. The intermediate allele frequencies at both *Pep-1* and *Sod-1* seem to reflect the geographic origin.

General conclusions

What practical consequences arise from these findings? Does the widely diminished gene pool pose a serious threat to the survival of the species? Evaluation of phenotypic traits, fitness parameters, mortality, fertility and fecundity do not reveal any severe effects caused by the low level of variation (Arnold 1990a). A low amount of variation could also lead to a limited repertory of defense against parasites or viral and bacterial infections. Although Alpine marmots harbour a number of different parasites (Bergmann and Prosl 1988, Manfredi *et al.* 1992, Preleuthner 1992), there seems to be no evidence that these infections are able to elicit overt pathological symptoms (Jettmar and Anschau 1951, Gossow and Dieberger 1989, Lichtenstein 1989, Prosl *et al.* 1992). In summary, the Alpine marmot populations are still genetically well equiped to cope with the present environmental regime. Little genetic plasticity, however, may become a problem

if the populations were exposed to major ecological perturbations like gross climatic changes (e.g. greenhouse effect), deterioration of the natural habitat or ascendancy of new pathogens. Therefore it seems important to preserve the still available genetic variety of *M. marmota*. Foundation of new populations and introduction of additional individuals into already colonized areas should take into account that only autochthonous populations harbour the full genetic spectrum of the species, whereas introduced populations have further diminished their already limited genetic reservoir by founder effects and drift. For example, the data for *Sod-1* indicate a trend for elimination of the slow allele. Release from introduced populations, as it has been repeatedly done in the Eastern Alps, can cause further reduction of variety that may on the long run lead to destruction of the adaptive potential.

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