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Genetic variation in two alpine populations of chamois, *Rupicapra rupicapra* L.

By Christine Miller and G. B. Hartl

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Abstract

Genetic variation in 62 chamois (*Rupicapra rupicapra* L.) from two alpine populations was investigated by horizontal starch gel electrophoresis. 25 enzymes were examined in kidney tissues. Of 41 loci scorable, 8 were polymorphic. Indices of genetic variability show considerable higher values than in previous studies on the population genetics of chamois. These results are discussed with respect to data on the genetic variability found in other large mammalian species.

Introduction

The chamois (*Rupicapra rupicapra* L.) are one of the rare wildlife species, which are thriving in their environment nowadays. From past overhunting and scattered distribution they have recovered and are now flourishing, some populations even experiencing various degrees of overpopulation. Chamois can live in a variety of different habitate types from low mountain ranges to high alpine regions.

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In contrast to other wild living ungulates like red deer (Gyllensten et al. 1983), moose (Ryman et al. 1977, 1980), and white-tailed deer (Manlove et al. 1975, 1976; Ramsey et al. 1979; Baccus et al. 1983) the genetic constitution of chamois populations is largely unknown. An initial study of three alpine populations from Northern Italy by Nascetti et al. (1985) revealed low genetic variability in *R. rupicapra rupicapra* in comparison to *R. rupicapra pyrenaica* and *R. rupicapra ornata* without an obvious explanation. Therefore we started an extensive research programme in chamois, to evaluate the genetic constitution of populations living under different environmental conditions and with different population histories. In this study we investigated two alpine populations showing considerable genetic variability.

Material and methods

Samples of kidney from 62 chamois representing two different populations were collected by local hunters during the hunting season in 1984, most of them between the end of october and the end of december.

55 specimens were taken from the Mangfall-Alps in Bavaria. This mountain range is part of the Northern Chalciferous Alps, and stretches from the Inn- to the Isar-River along the Austrian boarder. The highest summits do not exceed 1885 m. From this autochthonous chamois population 17 females, 22 males from 3 to 15 years of age, 11 yearlings, 5 kids, and one 3 year old animal with unidentified sex were taken. The sample included 2 mother-offspring pairs. There is reported migration of chamois throughout the whole area. So all the Bavarian stocks from which we obtained samples are supposed to form a single population with a considerable amount of panmixis. The second sample consisting of 4 females and 3 males, all adult specimens, came from a population in Lower Austria, 20 km west of Baden (15° 15′ east, 47° 55′ north). The highest elevation in the pre-alpine chamois range is the Kienjoch with 1106 m. The samples were taken from freshly killed animals and stored at −20 °C in deepfreezers at the hunters'. Cold winter weather during the sampling period prevented considerable protein denaturation even when the samples were frozen several hours after death of the specimens. Hunters donating samples were requested for information on:

- area and month of the hunt
- sex and age of the specimens
- eventual mother-offspring pairs shot.

Stored on dry ice the samples were brought to the laboratory and kept there in the deepfreezer until electrophoresis. Homogenates were prepared according to Csaikl et al. (1980) using an Ultra Turrax TP 18/10. The samples were subjected to horizontal starch gel electrophoresis using the following buffer systems: a phosphate buffer (buffer system I), and a tris-maleate buffer (buffer system II), both described in Csaikl et al. (1980), and a tris-citrate buffer (buffer system III), described in Manlove et al. (1975).

Buffer system I was used for LDH, 6-PGD, G-6-PD, SOD, PGM, ES, PEP, MPI and GPI, buffer system II for α-GPDH, SDH and ACY-1, buffer system III for MDH, ME, IDH, GDH, GLUD, CAT, AAT, HK, CK, AK, ACP, ADA and FH. After electrophoresis the gels were sliced and stained for enzyme activity as cited in Hartl and Csaikl (in press), with the following exceptions: for HK and CK according to Shaw and Prasad (1970), for AK and FH according to Siciliano and Shaw (1976), and for PEP according to Siciliano and Shaw (1976) using L-leucyl-L-alanine as substrate.

The genetic interpretation of the banding patterns was based on the principles described by Harris (1980) and Harris and Hopkinson (1976). Furthermore the published enzyme structure scheduled by Darnall and Klotz (1976) was used for the interpretation of heterozygotes. The results were compared to electrophoretic patterns in wild boars as described in Hartl and Csaikl (in press). The most common allele in chamois was designated arbitrarely "100"; variant alleles according to their relative mobility.

Results

Screening of 25 enzyme systems representing a minimum of 43 genetic loci revealed polymorphism in 8 isoenzymes: malic enzyme-1 (ME-1), catalase (CAT), hexokinase-1 (HK-1), phosphoglucomutase-2 and -3 (PGM-2, PGM-3), acid phosphatase-1 and -3 (ACP-1, ACP-3) and adenosine deaminase (ADA). The isoenzymes α-GPDH-1, -2; SDH, LDH-1, -2; MDH-1, -2; ME-2; IDH; 6-PGD; GDH; G-6-PD; GLUD; SOD-1, -2; AAT-1, -2; HK-2; CK; AK-1, -2; PGM-1; ES-1, -2, -3 (unscorable); ACP-2; PEP-1, -2,

Table

List of polymorphic loci (99 % criterion) in chamois

Enzyme locus	Allele	Bavaria (n = 55) p	H	E	n	Lower Austria (n = 7) p	H	E	n
ME-1	100	0.954	0.100	+	55	1.0	0.0		7
	123	0.046							
CAT	100	0.632	0.471	+	53	0.642	0.714	−	7
	140	0.368				0.358			
HK-1	100	0.847	0.217	+	46	0.714	0.285	+	7
	166	0.153				0.286			
PGM-2	100	0.518	0.563	+	55	0.500	0.714	+	7
	112	0.482				0.500			
PGM-3	100	0.858	0.195	+	46	1.0	0.0		7
	108	0.142				0.0			
ACP-1	100	1.0	0.0		53	0.714	0.571	+	7
	333	0.0				0.286			
ACP-3	100	0.971	0.056	+	53	1.0	0.0		7
	110	0.029				0.0			
ADA	100	0.764	0.313	+	51	1.0	0.0		7
	114	0.236				0.0			
	L = 41	$\overline{P}$ = 0.170	$\overline{H}$ = 0.046			$\overline{P}$ = 0.097	$\overline{H}$ = 0.065		
			$\overline{A}$ = 1.170				$\overline{A}$ = 1.097		

n = sample size, p = allele frequency, H = heterozygosity, E = Hardy-Weinberg equilibrium (+ in agreement/$p > 0.05$, − not in agreement/$p < 0.05$ with expected heterozygosity), $\overline{P}$ = proportion of polymorphic loci, $\overline{H}$ = average heterozygosity, L = number of loci scored, $\overline{A}$ = mean number of alleles per locus.

-3; ACY-1; FH-1, -2; MPI; GPI-1, -2 were monomorphic in both populations. The allele frequencies and heterozygosity at all polymorphic loci are given in the Table.

The malic enzyme exhibits two anodally migrating isoenzymes ME-1 and ME-2, representing the gene products of two different loci. Within the Bavarian sample heterozygotes were found at the more cathodal locus called ME-1 (Fig. 1). Polymorphism at this locus has also been detected by Nascetti et al. (1985) in chamois from Northern Italy. Within the Austrian sample the locus is monomorphic, showing only homozygotes for the most common allele in Bavaria. The catalase isoenzymes migrate anodally, but are poorly resolved. However, after several electrophoretic runs, where identical patterns were observed, an allelic interpretation seemed possible to us. The CAT locus was polymorphic in both populations studied. Both homozygotes and the corresponding heterozygote phenotype could be scored (Fig. 2). In hexokinase two anodally migrating isoenzymes HK-1 and HK-2 were found, representing the molecular products of two genetic loci. In HK-1 a polymorphism could be detected in both populations (Fig. 3). HK-2 might be polymorphic as well, but the banding patterns were not consistently scorable. Therefore we excluded this locus from the calculations. In PGM three anodally migrating fractions were detected similar to the pattern found in wild boars by Hartl and Csaikl (in press). Following the interpretation of Harris (1980) we identified them as the products of three genetic loci. In the least anodal fraction representing PGM-1 no genetic variability occured. PGM-2 revealed polymorphism in both populations (Fig. 4). In PGM-3 a variant allele was found only within the Bavarian sample (Fig. 4). This enzyme was previously reported to be monomorphic in chamois by Nascetti et al. (1985). On gels stained for ACP three

anodally migrating bands were found. For variants occured independently in all three isoenzymes we interpreted them as the gene products of three different loci. According to the banding patterns of heterozygotes ACP-1 and ACP-3 are dimers, whereas ACP-2 is monomeric. Polymorphism was detected in ACP-1 only within the Austrian and in ACP-3 only within the Bavarian sample. In ACP-2 one specimen from Bavaria showed a heterozygous phenotype (Fig. 5). In ADA one structural locus was found segregating for two alleles in the Bavarian population (Fig. 6). No variability at this locus occured in the Austrian population as well as in chamois from Northern Italy (Nascetti et al. 1985). Although no polymorphism could be detected, the banding patterns of esterases need complementary discussion. On gels stained for ES activity one cathodally and two anodally migrating fractions were found (Fig. 7). The cathodal fraction (ES-1) and the least anodal fraction (ES-2) seem to be the products of single loci without genetic variation. The most anodal fraction (ES-3) consists of several bands probably encoded by more than one genetic locus. Within the present material they could not be consistently scored and were excluded from the calculations. The enzymes not discussed individually showed always a picture typically for a homozygous state.

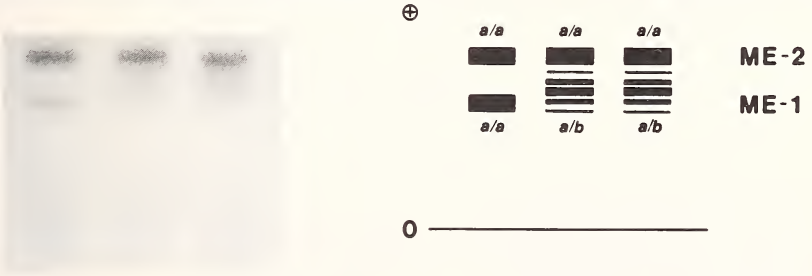


Fig.1 ME

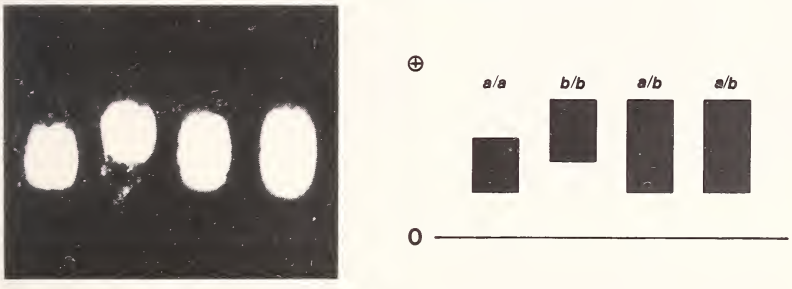


Fig.2: CAT

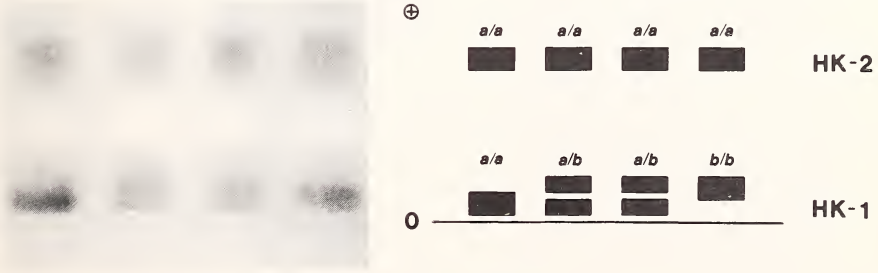
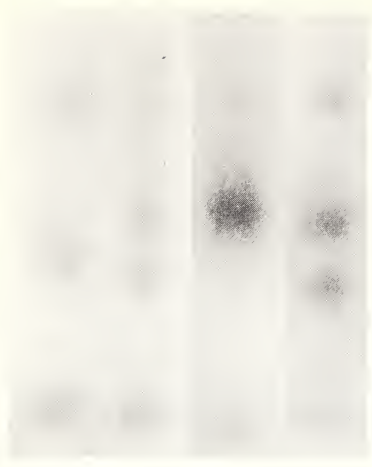


Fig.3: HK



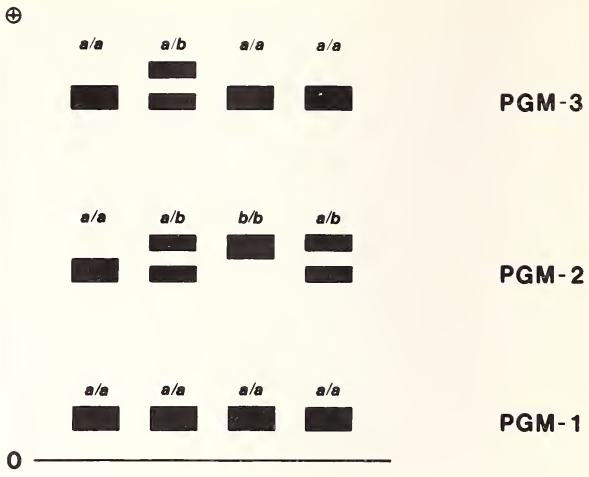


Fig. 4: PGM

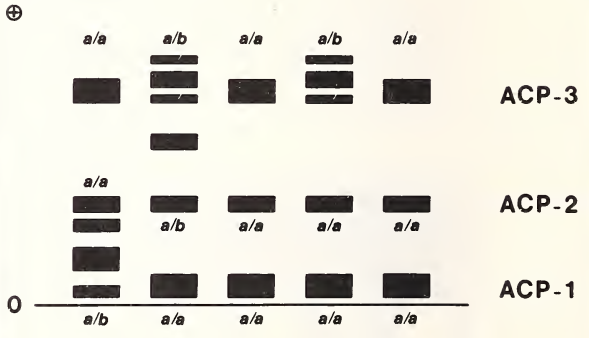


Fig. 5: ACP

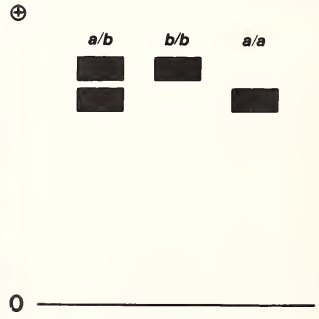


Fig. 6: ADA

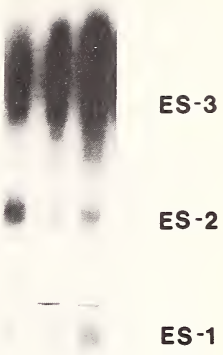


Fig. 1–7. Electrophoretic and diagramatic representation of malic enzyme (ME), catalase (CAT), hexokinase (HK), phosphoglucomutase (PGM), acid phosphatases (ACP), adenosine deaminase (ADA) and esterases (ES)

Fig. 7: ES

Discussion

The proportion of polymorphic loci averaged over both populations studied is similar to the value found in alpine chamois by Nascetti et al. (1985). In contrast average heterozygosity is considerable higher within our populations. Our results indicate, that *R. rupicapra rupicapra* is not less variable than *R. rupicapra pyrenaica* as stated by Nascetti et al. (1985). As discussed in Hartl (1985) the genetic variability found in a population or species depends strongly on the set of enzymes studied. Most loci which were found to be polymorphic in our populations (CAT, HK-1, PGM-3, ACP-1, -3) were not investigated by Nascetti et al. (1985). Therefore the populations in Northern Italy may be much more variable than described previously. Additionally genetic divergence calculated according to Nei (1972) is larger between the Austrian and the Bavarian population of *R. rupicapra rupicapra* ($\overline{D} = 0{,}0046$), than between the three populations of Northern Italy examined by Nascetti et al. (1985) (average $\overline{D} = 0{,}0007$, calculated from the data given by Nascetti et al. 1985). This can be explained by a large amount of gene flow between the populations of the Tarvisio Reserve, the Belvisio Reserve and the Gran Paradiso National Park in the Italian Alps in contrast to the different origin of our populations and the reproductive isolation between them. Whereas average heterozygosity is rather equal, a remarkable difference was found in the extent of loci polymorphic between the Austrian and the Bavarian population studied. This result can be explained by different population histories. The Bavarian population represents an autochthonous stock living in the species' natural habitate range. Population size is large and there are no indications of a recent or past bottleneck situation. The Austrian population, however, roots back to a few chamois released at the end of the last century in a lowhill area not ranging above 900–1000 m. A considerable amount of genetic variation may have been lost at that time due to a genetic founder effect and random genetic drift. As stated by Nei et al. (1975) the proportion of polymorphic loci will be much more affected by a past bottleneck in population size than average heterozygosity. While the population grew, it colonized new areas. Now it is in connection with the autochthonous alpine populations in the South. Thus a certain amount of gene flow may also have contributed to an increase of genetic variation.

By comparison with indices of genetic variability in other large mammals as summarized in Baccus et al. (1983) and Hartl (1985), the chamois are likely to be one of the most variable species.

To evaluate possible environmental factors affecting genetic variability and to get a comprehensive picture of genetic relationships between alpine stocks of chamois comparative screenings of samples out of different regions and habitate types are at work.

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Zusammenfassung

Genetische Variabilität zweier alpiner Gemsenpopulationen, Rupicapra rupicapra L.

Die genetische Variabilität von 62 Gemsen (*Rupicapra rupicapra* L.) aus zwei alpinen Populationen wurde mit Hilfe von horizontaler Stärkegelelektrophorese ermittelt. Dazu wurden 25 Enzymsysteme aus Nierengewebsextrakten untersucht. Von 41 auswertbaren Genloci zeigten 8 einen Polymorphismus. Die Maße für genetische Variabilität zeigen zum Teil erheblich höhere Werte als in vorangegangenen Studien über die Alpengams. Diese Resultate werden im Zusammenhang mit dem Ausmaß an genetischer Variabilität bei anderen Großsäugern diskutiert.

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WISSENSCHAFTLICHE KURZMITTEILUNGEN

Chronic confrontation induces behavioral changes in dominant *Tupaia belangeri*

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Dominance and its physiological consequence can be studied in male *Tupaia belangeri* submitted to different types of social stress. Under chronic confrontation not only distinct physiological differences can be observed between winners and loosers, but in addition in the loosers variations occur between the subdominant and submissive animals, in many different physiological parameters (v. Holst et al. 1983). Unfortunately, no quantitative data are available describing behavior and its possible changes that occur during chronic confrontation. In order to obtain an insight into the ethological changes during chronic social stress in *Tupaia belangeri*, the following experiments were designed:

Two adult male *Tupaia belangeri* (♂ 447, ♂ 525) were kept in separate rooms, (26 ± 1 °C, 60 ± 7 % rel. humidity) in identically equipped cages (100 × 80× 124 cm in size) with horizontally and diagonally orientated branches. Individual behavior was recorded on video tapes during the first three hours of the light phase of an artifical L:D (9 am: 9 pm) on 3 days over a period of 3 months. After this, each animal was confrontated in his home cage with a strange conspecific ("intruder") over a period of 17 days. On day 1, 2, 3, and 10 of the confrontation, behavior was recorded for three hours after the beginning of the light phase. Nineteen days after the end of the confrontation, the animal's behavior was monitored again. During the experiments, the body weight of both experimental animals and of the "intruder" was recorded daily. Thirty-five defined variables of behavior (from the categories "locomotion", "comfort-activities", "investigative-behavior", "behavior related to metabolism", "territorial-behavior", definitions according to Richarz 1976) were associated with their spatial distribution in a coordinate system drawn over the cages. A total of 48 observation hours were analysed, providing more than 210 000 units of information. According to their body weight changes, the "intruder" was classified as subdominant (mean loss 10,5 %) whereas the cage-"owners" showed a more or less stable