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Spatial partitioning of allozyme variability in European mountain hares (*Lepus timidus*): gene pool divergence across a disjunct distributional range?

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Abstract

To investigate if the postglacial dispersion of mountain hares (*Lepus timidus*) into the present geographically separated ranges in Europe has produced marked gene pool differentiation, 209 individuals from Scandinavia, Russia, the Alps, Scotland, and Ireland were screened for allozymic variability at 40 structural gene loci by horizontal starch gel electrophoresis. Polymorphisms were detected at 13 loci. Most alleles were identical with those of brown hares (*Lepus europaeus*) studied earlier in Europe. Average expected heterozygosity (2.0–5.0 %) and rates of polymorphism (8.8–29.4 %) in regions or subspecies were comparable to those of local samples of European brown hares studied earlier. Despite a high amount (31.3 %) of "private alleles", genetic distances (Nei's 1978 D: 0.000–0.008 among subspecies, and 0.000–0.017 among regions) were similar to those found among local samples of central European brown hares. This indicates low genetic differentiation among gene pools of subspecies or regions. Also, relatively low mean F_{ST} values (0.157 for regions, 0.14 for subspecies) and low numbers of significantly differing allele frequencies indicated little genetic differentiation. Wright's (1978) hierarchical F-statistics revealed that less than 1 % of the relative genetic variation was partitioned among subspecies but 13.6 % among regions within subspecies. All results conform to the hypothesis of a quite panmictic gene pool of late-glacial and postglacial mountain hares in Europe. They also support the view that no severe drift has occurred in postglacial populations during the colonization of the present ranges.

Key words: *Lepus timidus*, allozymes, colonization, genetic differentiation, disjunct distribution

Introduction

Mountain hares (*Lepus timidus*) have a disjunct distribution in Europe, with natural ranges in the subarctic/arctic regions of Russia and Fennoscandia, the Baltic region and Poland, the Alps, Scotland, and Ireland. Mountain hares from these regions are considered separate subspecies (*L. t. timidus*, *L. t. kozhevnikovi*, *L. t. sylvaticus*, *L. t. varronis*, *L. t. scoticus*, *L. t. hibernicus*), mainly due to morphometric differences and pelage coloration (see Angerbjörn and Flux 1995). According to late Pleistocene and early Holocene

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ecotopes, geography, and fossil records (e.g., LANG 1994; STUART 1982; DÖPPES 1997; see also CORBET 1986), however, this hare species was most probably continuously distributed over large parts of Europe between the northern and the Alpine ice sheets by the end of the last glaciation period (at 10.000–12.000 YBP). Apparently, they were hunted by Magdalénien Cro-Magnon people of central Europe (e.g., DÖPPES 1997).

This study addresses the degree of cross gene pool differentiation among the currently spatially well separated subspecies of European mountain hares. Provided the late Pleistocene population of mountain hares did exhibit a panmictic gene pool across large parts of central and north-central Europe, rather than an already substructered one, and no severe or long lasting demographic bottlenecks ("founder effects" etc.) have occurred during the postglacial colonization of the present ranges, we should expect a low gene pool differentiation among the currently acknowleged subspecies in Europe. Alternatively, a possible structuring of the late glacial gene pool into regional populations and/or strong genetic drift during the post-glacial colonization period might have led to signifcant genetic differences among the subspecies in Europe.

Material and methods

A total of 209 mountain hares was collected at diverse localities in the Alps (Switzerland, France, Austria), Scandinavia, the Ural mountains (Russia), Scotland (U.K.), the Irish Republic, and Northern Ireland (U.K.) between 1994 and 1996. Detailes of sampling localities and sample sizes are given in figure 1. These hares can be allocated to four nominal subspecies: *L. t. timidus* (Scandinavia, Ural), *L. t. varronis* (Alps), *L. t. scoticus* (Scotland), and *L. t. hibernicus* (Ireland). The sample from Scotland (Mull) may also include the subspecies *L. t. hibernicus*, or *L. t. scoticus* × *L. t. hibernicus* hybrids because of the introduction of this subspecies to Mull in the last century (CORBET and SOUTHERN 1977; see also FLUX 1970 for mountain hares from mainland Scotland). Among the presently studied mountain hares from Sweden (*L. t. timidus*) introgression of *L. t. sylvaticus* cannot be entirely excluded. The Swedish hares were shot in January/February but unfortunately the hunters did not make any special remarks as to blue/grey coat colour which is typical for *L. t. sylvaticus* (BERGENGREEN 1969).

Sexing of hares was carried out by inspection of their internal reproductive organs. Age (adult vs. juvenile/subadult) was estimated by body size, body weight, and by checking for the occurrence of the lateral epiphyseal protrusion of the ulna; the latter method separates juveniles/subadults (born in the last reproductive season) from older ones (WALHOVD 1965).

The following 25 isozymes/-systems encoded by 40 hypothetical structural gene loci were assayed for allozymic variation by horizontal starch gel electrophoresis (isozyme/-system, abbreviation, E.C. number, and corresponding structural gene loci in parentheses): alpha-glycerophosphate dehydrogenase (GDC, 1.1.1.8, Gdc), sorbitol dehydrogenase (SDH, 1.1.1.14, Sdh), lactate dehydrogenase (LDH, 1.1.1.27, Ldh -1, -2), malate dehydrogenase (MOR, 1.1.1.37, Mor -1, -2), malic enzyme (MOD, 1.1.1.40, Mod -1, -2), isocitrate dehydrogenase (IDH, 1.1.1.42, Idh -1, -2), 6-phospho-gluconate dehydrogenase, (PGD, 1.1.1.44, Pgd), glutamate dehydrogenase (GLUD, 1.4.1.3, Glud), catalase (CAT, 1.11.1.6, Cat), superoxide dismutase (SOD, 1.15.1.1, Sod -1, -2), purine nucleoside phosphorylase (NP, 2.4.2.1, Np), aspartate amino-transferase (AAT, 2.6.1.1, Aat -1, -2), hexokinase (HK, 2.7.1.1, Hk -1, -2, -3), creatine kinase (CK, 2.7.3.2, Ck -1, -2), adenylate kinase (AK, 2.7.4.3, Ak -1, -2), phospho-glucomutase (PGM, 2.7.5.1, Pgm -2, -3), esterases (ES, 3.1.1.1, Es -1; ES-D, 4.2.1.1, Es-D), acid phosphatase (ACP, 3.1.3.2, Acp -1), fructose-1,6-diphosphatase (FDP, 3.1.3.11, Fdp-1), β-galactosidase (β-GAL, 3.2.1.23, β-Gal), peptidases (PEP, 3.4.11, Pep -1, -2), fumarate hydratase (FH, 4.2.1.2, Fh), aconitase (ACO, 4.2.1.3, Aco -1, -2), mannose phosphate isomerase (MPI, 5.3.1.8, Mpi), glucose phosphate isomerase (GPI, 5.3.1.9, Gpi -1, -2).

Tissue preparation, electrophoresis and protein-specific staining followed GRILLITSCH et al. (1992). Allelic variants were resolved by direct side-by-side comparison of migrating allozymes, including five brown hares (*Lepus europaeus*) on the same gels. For designation of alleles we used the nomenclature of GRILLITSCH et al. (1992). Genotypes at polymorphic loci were determined in each specimen according to the principles of enzyme electrophoresis (e.g., RICHARDSON et al. 1986; ROTHE 1994). In several individuals, however, genotypes could not be determined for the entire set of loci due to insufficient

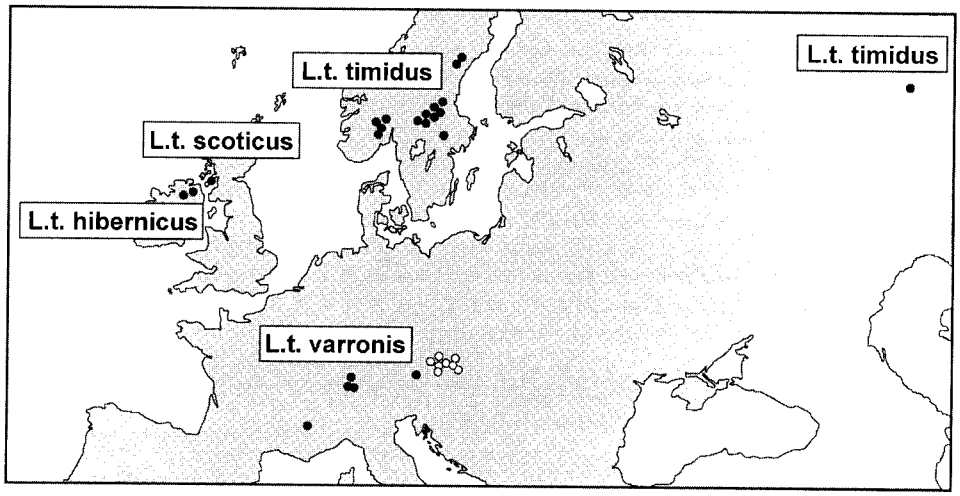

Fig. 1. Sampling locations of mountain hares (full circles) and associated subspecies names. Sample sizes in parentheses. Switzerland: canton Grisons, central and northern parts (49); Engadin (23); Val Mesolcina, Val Calanca, Val Bregaglia, Val Poschiavo (15); canton Glarus (16); Austria: Hohe Tauern (4); France: St. Véran (3); Abriès (3); Châteauroux (1); Aiguilles (1); Sweden: Jämtland (22); Växvik region (6); Väster- and Norbotten (7); Uppland (7); Norway: Ringebu (19); South Norway (5); Telemark (5); Russia: Polevskoy, Ural (14); Scotland (U.K.): Mull (5); Northern Ireland (U.K.): Antrim (1); Tyrone (1); Rep. Ireland: Mayo (1); Sligo (1). Open circles: Local populations of brown hares (*Lepus europaeus*) from Austria (cf., HARTL et al. 1993) used for comparison of genetic differentiation.

quality of resolution producing ambiguous interpretations. All population genetic statistics regarding regional samples of *L. timidus* and the comparison of *L. timidus* regional samples and the *L. europaeus* local samples were based on 40 loci. For the comparison of *L. timidus* subspecies all analyses were based on 34 loci (omitting the Mor-2; Ck-1, -2; Pgi; Cat; Gdc loci) because of total lack of data for these loci in certain subspecies.

Allele frequencies were calculated by using the BIOSYS-1 pc package 1.7 (SWOFFORD and SELANDER 1989). Allele frequencies of hares from Switzerland were tested for independence of age class (young of the year vs. older animals) or sex by Fisher's exact tests (using SPSS). Association of genotypes between loci was also tested by Fisher's exact tests for each pair of polymorphic loci with aggregated genotypes to check for linkage disequilibrium. Significance was based on sequential Bonferroni procedures (with a nominal alpha = 0.05) to account for multiple testing (RICE 1989). Allele frequencies at single loci were tested for significant variation between pairs of regions and pairs of subspecies by Fisher's exact tests of aggregated alleles in cases of more than two alleles per locus and sequential Bonferroni procedure.

The BIOSYS-1 pc package, release 1.7 (SWOFFORD and SELANDER 1989) was also used to calculate the rate of polymorphism (P, 99 % criterion), the mean number of alleles per locus (A), and mean heterozygosities (H_e-expected, H_o-observed) for each regional, subspecies, and local sample. It was further employed to calculate WRIGHT's (1978) non-hierarchical and hierarchical F-statistics. The latter was calculated to test for partitioning of genetic variability among subspecies relative to partitioning among regions within subspecies. NEI's (1978) genetic distances, corrected for small sample sizes, ROGERS' (1972) distances and modified ROGERS' distances (WRIGHT 1978) between all pairs of regional and subspecies samples of mountain hares and local samples of brown hares were calculated. Regarding brown hares, eight local samples studied earlier in the same laboratory (HARTL et al. 1993) were used with adjusted numbers of loci (n = 40). Relationships of pairwise genetic distances were revealed by an unrooted Wagner dendrogram (FARRIS 1972).

Results

Polymorphism was revealed at 13 loci. The overall rate of polymorphism (99 % criterion, 40 loci considered) for European mountain hares amounted to 32.5 %. Polymorphic loci, alleles, and associated allele frequencies are given in table 1 for four regions of Europe (disregarding subspecific allocation), and in table 2 for subspecies. Allele frequencies of mountain hares from Switzerland did not vary significantly among age classes or sexes. Values of

Table 1. Allele frequencies (%) at polymorphic loci of mountain hares from four regions of Europe based on 40 loci. H_o = average observed heterozygosity, H_e = average expected heterozygosity, P = rate of polymorphism (99% criterion), A = mean number of alleles per locus. Significant deviations of genotype frequencies from expected Hardy-Weinberg frequencies are indicated for respective loci and regions ([a] $p < 0.05$; [b] $p < 0.01$, significance tests using exact probabilities).

Subspecies / Locus	Allele	Scandinavia (n = 74) *L. t. timidus*	Alps (n = 112) *L. t. varronis*	Ural (n = 14) *L. t. timidus*	NW Europe (n = 9) *L. t. scoticus* and *L. t. hibernicus*
Sdh	a	0.000	0.041[b]	0.000	0.111
	b	1.000	0.959	1.000	0.889
β-Gal	a	0.000	0.016	0.400[b]	0.000
	b	1.000	0.964	0.600	0.100
	c	0.000	0.020	0.000	0.000
Ldh-2	a	1.000	1.000	1.000	0.889
	d	0.000	0.000	0.000	0.111
Idh-2	a	0.831[a]	0.925	0.321	1.000
	d	0.169	0.071	0.679	0.000
Pgd	a	1.000	1.000	0.964	1.000
	b	0.000	0.000	0.036	0.000
Hk-2	a	0.973	1.000	1.000	1.000
	b	0.027	0.000	0.000	0.000
Es-1	a	0.143[b]	0.100	0.143	0.071
	b	0.843	0.900	0.857	0.786
	c	0.000	0.000	0.000	0.143
	e	0.014	0.000	0.000	0.000
Es-D	a	0.810	0.887	0.929	0.688
	b	0.148	0.113	0.071	0.312
	c	0.042	0.000	0.000	0.000
Pep-2	a	0.036	0.054	0.000	0.000
	b	0.906	0.922	1.000	1.000
	c	0.058	0.024	0.000	0.000
Acp-1	a	0.000	0.036[b]	0.000	0.000
	b	1.000	0.964	1.000	1.000
Mpi	a	0.948	0.964	0.929	0.938
	b	0.052	0.014	0.071	0.062
	c	0.000	0.022	0.000	0.000
Acon	a	0.979	0.986	1.000	1.000
	b	0.021	0.014	0.000	0.000
Me-2	a	1.000	1.000	0.929[a]	1.000
	b	0.000	0.000	0.071	0.000
H_o		0.026	0.021	0.025	0.034
H_e		0.031	0.024	0.041	0.032
P		17.5	22.5	17.5	12.5
A		1.3	1.3	1.2	1.1

Table 2. Allele frequencies (%) at polymorphic loci of four subspecies of mountain hares from Europe based on 34 loci. H_o = average observed heterozygosity, H_e = average expected heterozygosity, P = rate of polymorphism (99 % criterion), A = mean number of alleles per locus. Significant deviations of genotype frequencies from expected Hardy-Weinberg frequencies are indicated with the "a" allele for respective loci and subspecies ([a] $p<0.05$; [b] $p<0.01$, significance tests using exact probabilities).

Subspecies / Locus	Allele	*L. t. timidus* (n = 88)	*L. t. varronis* (n = 112)	*L. t. scoticus* (n = 5)	*L. t. hibernicus* (n = 4)
Sdh	a	0.000	0.041[b]	0.100	0.125
	b	1.000	0.959	0.900	0.875
β-Gal	a	0.103[b]	0.016	0.000	0.000
	b	0.897	0.964	1.000	1.000
	c	0.000	0.020	0.000	0.000
Ldh-2	a	1.000	1.000	1.000	0.750
	d	0.000	0.000	0.000	0.250
Idh-2	a	0.774[b]	0.929	1.000	1.000
	d	0.256	0.071	0.000	0.000
Pgd	a	0.994	1.000	1.000	1.000
	b	0.006	0.000	0.000	0.000
Hk-2	a	0.977	1.000	1.000	1.000
	b	0.023	0.000	0.000	0.000
Es-1	a	0.143[a]	0.100	0.000	0250
	b	0.844	0.900	0.800	0.750
	c	0.000	0.000	0.200	0.000
	e	0.013	0.000	0.000	0.000
Es-D	a	0.829	0.887	0.900	0.333
	b	0.135	0.113	0.100	0.667
	c	0.035	0.000	0.000	0.000
Pep-2	a	0.030	0.054	0.000	0.000
	b	0.922	0.922	1.000	1.000
	c	0.048	0.024	0.000	0.000
Acp-1	a	0.000	0.036[b]	0.000	0.000
	b	1.000	0.964	1.000	1.000
Mpi	a	0.944	0.964	1.000	0.833
	b	0.056	0.014	0.000	0.167
	c	0.000	0.022	0.000	0.000
Acon	a	0.983	0.986	1.000	1.000
	b	0.017	0.014	0.000	0.000
Me-2	a	0.989[b]	1.000	1.000	1.000
	b	0.011	0.000	0.000	0.000
H_o		0.030	0.025	0.024	0.066
H_e		0.044	0.029	0.020	0.050
P		29.4	26.5	8.8	14.7
A		1.4	1.4	1.1	1.1

genetic variability for the regions (based on 40 loci) are listed in table 1, and for the subspecies (based on 34 loci) in table 2. The observed genotypic distributions differed significantly from Hardy-Weinberg expectations at six loci in three regional samples (Tab. 1) and at six loci in two subspecies samples (Tab. 2). Basically, all these significant genotype deviations were due to heterozygote deficiencies. Pairwise NEI's (1978) genetic distances in mountain hares, corrected for small sample sizes, ranged between 0.000–0.008 among subspecies, and between 0.000–0.017 among regions (Tab. 3). Modified Rogers' distances (WRIGHT 1978) ranged between 0.037–0.117 among subspecies, and between 0.024–0.135 among regions (Tab. 3). Pairwise genetic distances between single regional samples of mountain hares and

single local samples of central European brown hares ranged between 0.068–0.093 (NEI's 1978 D), and between 0.253–0.295 (Rogers' modified distances; WRIGHT 1978).

In mountain hares, locus-specific F_{ST} and F_{IS} values did not show any particular concordance across loci; for the regional samples mean $F_{ST} = 0.157$, mean $F_{IS} = 0.17$, and mean $F_{IT} = 0.3$. For the subspecies samples the respective values were 0.14, – 0.02, and 0.12. The relative genetic differentiation (F_{ST} values) for pairs of subspecies are listed in table 4 along with associated significances of heterogeneity of allele frequencies. Only

Table 3. NEI's (1978) genetic distances for small sample sizes (above diagonal) and modified Rogers' distances (below diagonal) among pairs of mountain hares from European regions (based on 40 loci), and subspecies (based on 34 loci). Regions: SCAN = Scandinavia, ALPS, URAL, NWE = Northwest Europe (Scotland, Ireland).

	SCAN (1)	ALPS (2)	URAL (3)	NWE (4)	*L. t. timidus* (5)	*L. t. varronis* (6)	*L. t. scoticus* (7)	*L. t. hibernicus* (8)
(1)	–	0.000	0.010	0.001				
(2)	0.024	–	0.013	0.001				
(3)	0.106	0.115	–	0.017				
(4)	0.049	0.047	0.135	–				
(5)					–	0.001	0.003	0.008
(6)					0.039	–	0.000	0.008
(7)					0.062	0.037	–	0.007
(8)					0.115	0.113	0.117	–

Table 4. F_{ST} values for pairs of subspecies (above diagonal) and significance values for heterogeneity of allele frequencies (below diagonal); significance is based on exact Fisher's test and sequential Bonferroni procedure (sig.: $p < 0.05$; n. s.: $p > 0.05$). Significance is given with significantly varying allele frequencies at least at one locus.

	L. t. timidus (1)	*L. t. varronis* (2)	*L. t. scoticus* (3)	*L. t. hibernicus* (4)
(1)	–	0.02	0.056	0.123
(2)	sig.	–	0.028	0.139
(3)	n. s.	n. s.	–	0.164
(4)	sig.	sig.	n. s.	–

Table 5. F_{ST} values for pairs of sampling regions of *L. t. varronis* and *L. t. timidus*, respectively (above diagonal), and significance values for heterogeneity of allele frequencies (below diagonal), based on exact Fisher's test and sequential Bonferroni procedures (sig.: $p < 0.05$; n. s.: $p > 0.05$). Significance is given if at least one locus shows significantly varying allele frequencies in a pairwise comparison.

L. t. varronis		(1)	(2)	(3)	(4)	(5)
	Switzerland (1)	–	0.146	0.048	0.173	0.014
	Austria (2)	n. s.	–	0.055	0.237	0.107
	France (3)	n. s.	n. s.	–	0.171	0.024
L. t. timidus						
	Ural (4)	sig.	n. s.	sig.	–	0.135
	Scandinavia (5)	n. s.	n. s.	n. s.	sig.	–

6.8 % of all possible pairwise comparisons of region-specific allele frequencies at polymorphic loci (59 tests) yielded significant differences. According to theory, significance of pairwise F_{ST}-values is given with allele frequencies varying significantly at least at one locus studied (WRIGHT 1978). F_{ST}-values for pairs of sampling regions within the subspecies *L. t. timidus* and *L. t. varronis*, respectively, as well as associated significance values for heterogeneity of allele frequencies are given in table 5. Significant differences of allele frequencies at polymorphic loci were found only in 5.4 % of all possible pairwise comparisons between regions (92 tests). Details of WRIGHT's (1978) hierarchical F-statistics giving the proportions of genetic variation partitioned among the four subspecies studied, relative to the regional effect on genetic partitioning, is presented in table 6. An unrooted Wagner dendrogram based on Rogers' distances, depicting genetic relationships among mountain hares from the four regions studied in Europe and eight local samples of brown hares from central Europe (HARTL et al. 1993) is presented in figure 2.

Table 6. Wright's hierarchical F-statistics in European mountain hares, based on 34 allozyme loci. Variance components and F-statistics combined across loci.

Comparison X	Comparison Y	variance component	F_{XY}
sampling regions[1]	subspecies	0.2096	0.129
sampling regions[1]	total variance	0.2230	0.136
subspecies	total variance	0.0134	0.008

[1] Austria, France, Ireland, Scandinavia, Scotland, Switzerland, Ural.

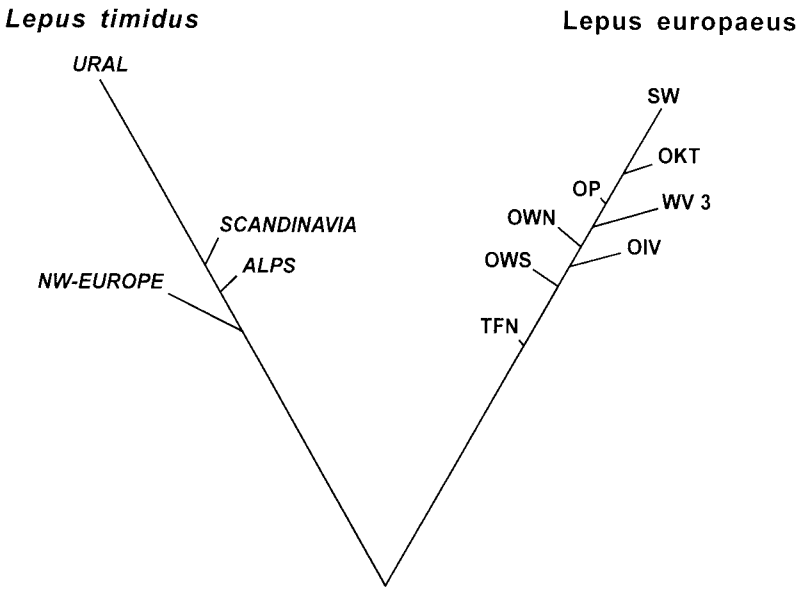

Fig. 2. Unrooted Wagner dendrogram (midpoint rooting of longest path) depicting genetic relationships among mountain hares (*Lepus timidus*) from various regions of Europe and brown hares (*L. europaeus*) from eight local samples of central Europe. The dendrogram is based on ROGERS' (1972) distances, calculated from allele frequencies at 40 loci. Total tree length = 0.192, distance between "Ural" and "Scandinavia" = 0.032, cophenetic correlation coefficient = 0.986

Discussion

The level of gene pool variability of European mountain hares, as indicated by allozyme heterozygosity, rate of polymorphism, and mean number of alleles per locus in the diverse regions and subspecies is similar to that of brown hares from various continental European regions (HARTL et al. 1989, 1990, 1992, 1993, 1995; SUCHENTRUNK et al. 1998, 1999). The presently found heterozygosity values are typical for undisturbed populations of terrestrial mammalian species of diverse orders (NEVO 1978; TIEDEMANN et al. 1996). The somewhat reduced rates of polymorphism and mean numbers of alleles per locus in Scotish and Irish mountain hares are likely due to the low sample sizes. The H_e : P rates within regions and subspecies ranged between 0.107–0.256. These values fall within the range of "undisturbed" populations (TIEDEMANN et al. 1996), indicating populations without genetic depletion e. g., due to severe bottlenecks or long-term low effective population size. Average numbers of alleles per locus (A) do not give any hint for depauperated gene pools in Scandinavia, the Ural or the Alps. The low A-values for mountain hares from Scotland and Ireland are most probably due to the low sample sizes for these regions.

The overall rate of polymorphism of the mountain hares (32.5 %) appears to be somewhat greater than in brown hares. Combining the data of HARTL et al. (1989, 1990, 1992, 1993, 1994, 1995) and SUCHENTRUNK et al. (1998, 1999) for brown hares from various regions of Europe yields an overall rate of polymorphism of 25.9 %. Adjusting the set of loci analysed in brown hares to the presently studied set (40 loci) results in a value of 27.5 % for brown hares. The still somewhat higher value of mountain hares is due to three polymorphic loci (Me-2, Acp-1, Acon). But these three loci are only marginally polymorphic with variant alleles occurring in one or two regions, respectively. Furthermore, the Acp-1^a allele occasionally found in some mountain hares from Switzerland may result from rare cases of hybridization (cf. e. g., BALDENSTEIN 1863; FRAGUGLIONE 1966; SCHRÖDER et al. 1987; THULIN et al. 1997 a). In general, most of the loci found polymorphic in brown hares (HARTL et al. 1990, 1992, 1993; SUCHENTRUNK et al. 1998; 1999) are also polymorphic in the presently studied mountain hares. Moreover, most of the loci with several alleles in brown hares (Es-1, β-Gal, Pep-2, Mpi) reveal several of these alleles in the mountain hares too.

These very similar allele patterns hamper differential diagnosis by allozymes between mountain and brown hares. When comparing allozyme patterns of brown and mountain hares GRILLITSCH et al. (1992) screened only few mountain hares from one region in Austria. With that small and regionally limited sample size they obviously have missed some polymorphisms in mountain hares. Their results suggested a differential diagnosis between these two species by three loci. However, the allelic differences at the β-Gus and the Pgm-1 loci between the two species (GRILLITSCH et al. 1992) could not be proven presently because of dubious zymograms. The present results suggest that, among the array of loci screened, only the Acp-1 locus has alleles alternately fixed in the two species, with occasional cases of introgressive hybridization in mountain hares from the Alps. However, at present no allozyme data of brown hares from regions of potential introgressive hybridization are available to substantiate this hypothesis. In Scandinavian mountain hares no hint of introgressive hybridization was found presently, although THULIN et al. (1997 a) reported presence of mountain hare mtDNA in brown hares from the Upland region.

Despite the relatively high amount of "private alleles" at several loci (31.3 % of all studied loci) in various regions or subspecies of mountain hares, overall genetic distances among gene pools of the diverse regions or subspecies are generally low in magnitude. Because of their generally low frequencies, "private alleles" do not greatly influence genetic differentiation. NEI's (1978) genetic D-values among subspecies are similar to those found among local samples of brown hares within central Europe (e. g., HARTL et al. 1989, 1990, 1992, 1993). However, brown hares also exhibit low genetic differentiaton even

across large geographic distances in Europe; this indicates a rather panmictic network and a lack of discernible populations (HARTL et al. 1990; SUCHENTRUNK et al. 1999). Only mountain hares from the Ural region and Ireland show a slightly increased genetic divergence to mountain hares from the other study regions or subspecies. In Ural mountain hares this slight separation is only based on significantly increased frequencies of the β-Gal^a and the Idh-2^d alleles. It might e.g., result from the large geographic distance between the Ural and the other regions or indicate introgression of gene pool elements of *L. t. kozhevnikovi*. According to OGNEFF (1929) this subspecies occurs in the south Ural (near Miass) some 150 km south of the presently studied collection site. However, skull dimensions and the external features of the presently studied individuals tend to conform with those of *L. t. timidus* of various parts of European Russia, rather than with those of *L. t. kozhevnikovi* (albeit there is little morphometric differentiation between the two subspecies in Russia; see OGNEFF 1929).

The slightly raised level of genetic separation of Irish mountain hares is particularly due to changes in allele frequencies at the Es-d locus and the presence of a "private allele" (Ldh-2^d) with a frequency of 25 %. However, significant changes in allele frequencies between *L. t. hibernicus* and the other subspecies was only found at the Pep-2 locus. In view of the small sample size from NW Europe, and the fact that Scottish mountain hares were only screened from the isle of Mull, where Irish mountain hares had been liberated in the last century, no conclusions regarding the genetic differentiation between these two subspecies can be drawn.

The generally low level of genetic differentiation between subspecies or regions of mountain hares is also indicated by the small proportion of relative genetic variability partitioned among subspecies or regions and particularly by the low proportions of significantly varying allele frequencies between pairs of subspecies (6.8 %) or regions (5.4 %). Moreover, while 13.6 % of the relative genetic variation are partitioned among regions within subspecies, less than 1 % is partitioned between the subspecies studied. This means that gene pool divergence is greater among sampling regions within *L. t. timidus* and *L. t. varronis*, respectively, than between all studied subspecies. Hence, no distinct gene pools of the studied subspecies can be identified. Also, a single mountain hare collected in the Primorje region of Far East Sibiria analysed in our laboratory did not reveal any new allele.

All results agree with sequence data demonstrating an admixture of mtDNA haplotypes in mountain hares from Scandinavia and other parts of Europe (THULIN et al. 1997 b) without separation in clear phylogeographic units. The present allozyme results also conform to the hypothesis of a rather panmictic gene pool of late- and postglacial populations in central Europe without any specific phyletic blocks. They are also in agreement with the view that there were no severe demographic bottlenecks, founder effects, long-term low effective population sizes, multiple regional extinctions etc. in post-glacial populations during the colonization of the present ranges. Obviously, separation of postglacial European mountain hares into several geographic ranges has not resulted in distinct gene pools; and there is very little measureable evolutionary divergence between the pools of coding genes in the phenotypically specified subspecies.

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Zusammenfassung

Räumliche Verteilung der Allozymvariabilität bei europäischen Schneehasen (Lepus timidus): Genpool-Divergenz in einem disjunkten Verbreitungsgebiet?

Die Allozymvariabilität von 209 Schneehasen (*Lepus timidus*) von Skandinavien, Rußland, den Alpen, Schottland und Irland wurde mittels horizontaler Stärkegelelektrophorese von 40 Strukturgenloci festgestellt, um zu prüfen, ob die postglaziale Besiedelung der heutigen disjunkten Schneehasen-Verbreitung in Europa zu einer markanten genetische Differenzierung geführt hat. Die meisten Allele an den 13 polymorphen Loci waren identisch mit den schon früher bei europäischen Feldhasen (*L. europaeus*) gefundenen. Durchschnittliche erwartete Heterozygotie-Werte pro Region bzw. Subspecies (2,0–5,0%) sowie die Polymorphieraten (8,8–29,4 %) entsprachen denen bei Feldhasen aus früheren Untersuchungen. Trotz der hohen Rate (31,3 %) an Allelen, die ausschließlich in einzelnen Regionen oder Subspecies vorkamen, lagen die genetischen Distanzen (NEI's D: 0,000–0,008 zwischen Subspezies, 0,000–0,017 zwischen Regionen) grundsätzlich im Bereich jener Werte, wie sie zwischen lokalen Feldhasen-Populationen in Mitteleuropa früher festgestellt wurden. Ebenso zeigten die relativ geringen mittleren F_{ST} Werte (0,157 zwischen Regionen; 0,14 zwischen Subspecies) sowie die geringe Zahl an signifikanten paarweisen Unterschieden von Allelfrequenzen eine geringe genetische Differenzierung zwischen den Regionen bzw. Subspecies an. Während 13,6 % der relativen genetischen Variabilität zwischen Regionen innerhalb von Subspecies verteilt waren, lag der entsprechende Wert für die Verteilung zwischen den Subspecies unter einem Prozent. Alle Ergebnisse entsprechen der Hypothese einer panmiktischen spät- bzw. postglazialen Population in Mitteleuropa und der Annahme, daß bei der Kolonisation der heutigen disjunkten Verbreitungsgebiete in Europa keine starke genetische Drift erfolgt ist.

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