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Original investigation

Little allozyme and mtDNA variability in brown hares (*Lepus europaeus*) from New Zealand and Britain – A legacy of bottlenecks?

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Abstract

We studied cross nuclear and mitochondrial gene pools of brown hares (*Lepus europaeus*) from three local populations in Britain and two in New Zealand, to test the hypothesis of reduced genetic variability in hares from New Zealand resulting from few founders originating from Britain. Multilocus allozyme electrophoresis of 52 protein loci and analysis of restriction fragment length polymorphisms of total mitochondrial DNA based on 16 hexanucleotid-cleaving restriction enzymes were employed in 119 and 36 hares, respectively. Observed and expected average heterozygosities, rates of polymorphism, average numbers of alleles per locus, Shannon-Weaver information indices of allelic diversity, as well as values of haplotype and nucleotide diversity were similar in all regional samples. But hares from both New Zealand and Britain had significantly lower genetic diversity than brown hares from continental Europe studied earlier. Thus, gene pool erosion likely occurred already in British hares, perhaps associated with their probable introduction in Roman times. Theoretically, the small number of alleles found in British brown hares could have been sampled by the few hares that were reported as having constituted the founder stock in New Zealand in the nineteenth century. As expected, rare alleles of British brown hares were absent in New Zealand. But drift had only a slight effect on the gene pool composition of hares in New Zealand.

Key words: *Lepus europaeus*, allozymes, mtDNA, genetic bottleneck

Introduction

In New Zealand, brown hares (*Lepus europaeus*) have higher rates of ovarian tumors and missing posterior upper molars (M^3) than in Europe (Flux 1965, 1980; Parkes 1988; Suchentrunk et al. 1992). This might result from low genetic variability as a consequence of a small number of founder individuals (Flux 1965). Historical documents suggest that brown hares released in New Zealand in the 19th century by various Acclimatization Societies were mostly taken from Phillip Island, Victoria, Australia (Lever 1985; Flux 1990). There, only six hares had built up a population of 200 individuals by 1865, few years after introduction (Flux 1990; Flux et al. 1990; see also Rolls 1969 and Lever 1985). In Australia, brown hares were probably first successfully introduced in 1859 by W. Lyall on the shores of Western Port Bay, Victoria (Lever 1985), and afterwards on Phillip Island (Mahood 1983). In February 1864 an-

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other nine hares were released into an enclosure near Geelong, Victoria by T. Austin, who imported them from England (cf. Lever 1985). All Australian hares are considered originating from Britain (cf., Flux 1990), but no details as to specific regions are given in the available literature (Lever 1985; Flux pers. com.). The exact numbers of hares that have successfully bred after their naturalization in Australia and New Zealand remain unknown. However, the list of importations to New Zealand presented by Lever (1985) suggests limited genetic variability in the founder gene pool (see also Flux 1990).

In this study we compared levels of genetic variability of hares from New Zealand and Britain, to test this genetic bottleneck hypothesis (Flux 1965). Theory and empirical findings (e. g., Fuerst and Maruyama 1986; Leberg 1992; Hartl and Pucek 1994; Tiedemann et al. 1997) predict a smaller effect of bottlenecks on multi-locus allozyme heterozygosity than on other indicators of allozymic variability, such as the rate of polymorphism (P), and mean number of alleles per locus (A). Allozyme heterozygosity may even increase after bottlenecks (e. g., Leberg 1992). Thus, we expected lower P- and A-values for hares from New Zealand than for British brown hares, whereas heterozygosities might be similar. Particularly alleles with low frequencies in British brown hares might have not been sampled by the few founders in New Zealand. Drift effects could have caused shifts in allele frequencies and consequently increased genetic divergence between hares from Britain and New Zealand. We also expected a pronounced reduction of variability in the mitochondrial DNA (mtDNA), because of the lack of recombination in this maternally inhereted genome in post-bottleneck populations (e. g., Giles et al. 1980; Lansman et al. 1981; Avise 1994; Avise and Hamrick 1996; see e. g., Gyllensten et al. 1991 for paternal inheritance of mtDNA). Brown hares from the British Isles are conventionally considered a separate subspecies (*L. e. occidentalis* De Winton, 1898; cf. Corbet and Southern 1977; Arnold 1993). They might be genetically somewhat distinct from mainland European brown hares. Therefore, we compared the present data with adjusted data sets of continental European brown hares published earlier (Hartl et al.1993; Suchentrunk et al. 2000).

Material and methods

Specimens studied

We studied 119 hares from two regions in New Zealand and three in Britain (Fig. 1). In New Zealand, hares were collected in the Wairarapa region (n = 32) of the North Island in September/October 1993 by J. E. C. Flux ("Landcare Research", Lower Hutt), and in the Harper/Avoca catchment (n = 28) on the South Island in October 1993 and March 1995 by J. Parkes ("Landcare Research", Christchurch) and F. Suchentrunk. In Britain, collections were organized by S. Tapper (The Game Concervancy Trust, Fordingbridge, England) in February 1995 in three regions (Wiltshire, southern England, n = 20; Loddington, Leicestershire, central England, n = 19; Duns, Aberdeenshire, Scotland, n = 20). Most hares were dissected by one of the authors (FS). They were sexed by inspection of the internal reproductive organs and aged by checking the lateral epiphysial protrusion of the ulna (Stroh's sign), which separates young of the year (< approx. 7–10 months) from older hares (Suchentrunk et al. 1991). Liver, kidney, and spleen tissue samples were frozen at –20 °C until further use.

Allozymic diversity

We screened allelic variation at 52 hypothetical structural gene loci in 119 hares by standard horizontal starch gel electrophoresis of the following isozymes/-systems (isozyme/-system, abbreviation, E.C. number. and corresponding structural gene loci in parentheses): α-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-phosphogluconate dehydrogenase (PGD, 1.1.1.44, Pgd), glucose dehydrogenase (GDH, 1.1.1.47, Gdh-2), glucose-6-phosphate dehydrogenase (GPD, 1.1.1.49, Gpd), glyceraldehyde-3-phosphate dehy-

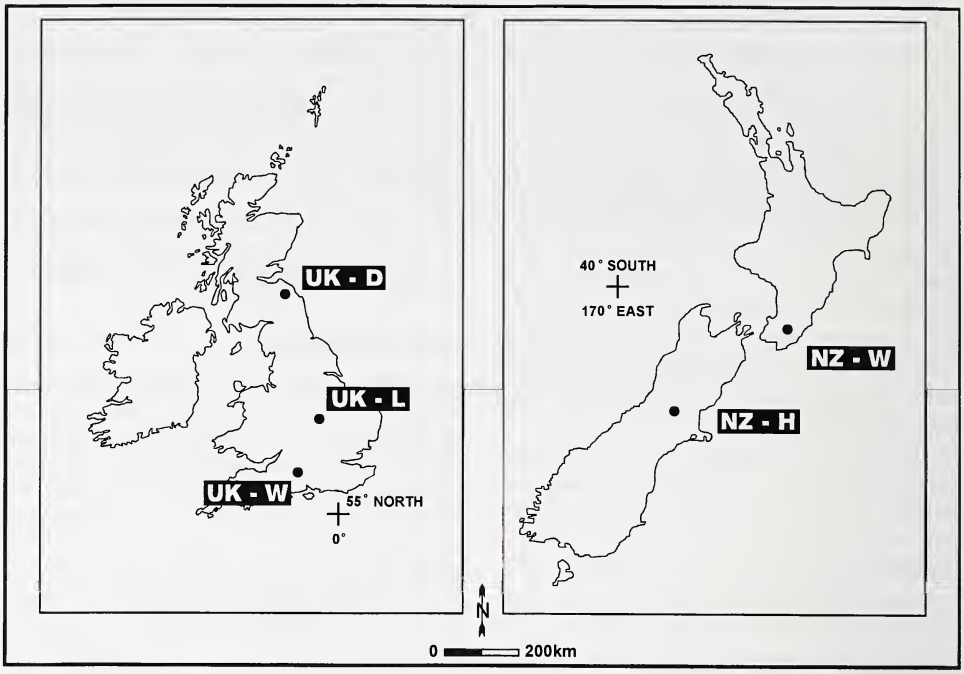

Fig. 1. Sampling regions of brown hares in Britain and New Zealand. Details are given in the text.

drogenase (GAPDH, 1.2.1.12, Gapdh), xanthine dehydrogenase (XDH, 1.2.3.2, Xdh), glutamate dehydrogenase (GLUD, 1.4.1.3, Glud), NADH-diaphorase (DIA, 1.6.2.2., Dia-1), 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 aminotransferase (AAT, 2.6.1.1, Aat-1,-2), hexokinase (HK, 2.7.1.1, Hk-1,-2,-3), pyruvate kinase (PK, 2.7.1.40, Pk-1), creatine kinase (CK, 2.7.3.2, Ck-1,-2), adenylate kinase (AK, 2.7.4.3, Ak-1,-2), phosphoglucomutase (PGM, 2.7.5.1, Pgm-1,-2,-3), esterases (ES, 3.1.1.1, Es-1; 4.2.1.1, Es-D), acid phosphatase (ACP, 3.1.3.2, Acp-1,-2,-3), fructose-1,6-diphosphatase (FDP, 3.1.3.11, Fdp-1), peptidases (PEP, 3.4.11, Pep-1,-2), guanine deaminase (GDA, 3.5.4.3, Gda), adenosine deaminase (ADA, 3.5.4.4, Ada-2,-3), aldolase (ALDO, 4.1.2.13, Aldo), 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). This suite of isozyme loci is identical to the loci screened by HARTL et al. (1993) for 20 regional samples of brown hares from central Europe, except for Acy-1 and β-Gal, which were not screened presently.

In tissue preparation, electrophoresis, and protein specific staining we followed GRILLITSCH et al. (1992). For resolving allelic variants we made direct side-by-side comparisons of migrating allozymes on the same gels by including samples of brown hares studied earlier in our laboratory, and adopted the allele nomenclature of HARTL et al. (1993) and SUCHENTRUNK (1993). We determined genotypes at polymorphic loci from zymograms according to principles of allozyme electrophoresis (HARRIS and HOPKINSON 1976; ROTHE 1994). In some individuals, however, we could not genotype all polymorphic loci due to poor resolution.

We used the BIOSYS-1 pc package, release 1.7 (SWOFFORD and SELANDER 1989) to calculate allele frequencies, average heterozygosity (H_o – observed, H_e – expected), proportion of polymorphic loci (P, 99% criterion), mean number of alleles per locus based on all 52 loci (A), and for exact tests of deviations of observed genotypes at polymorphic loci from Hardy-Weinberg expectations. As additional index of genetic diversity, we calculated the Shannon-Weaver information index (H′; see HEDRICK 1985) for each regional sample (RS) as the sum of locus-specific information indices based on allele frequencies.

We tested variation of locus-specific allele frequencies between pairs of regional samples by exact Fisher's tests and based significance decisions on Sequential Bonferroni procedure (Rice 1989), to account for multiple testing. Also, we based exact Fisher's tests of variation of allele frequencies between the two age classes (young of the year vs. older animals) and sex on Sequential Bonferroni procedure. In order to evaluate possible drift effects by e. g., founder events and/or long-term low effective population sizes, we calculated pairwise genetic D values (Nei 1978), modified Rogers' distances, and fixation indices (F_{ST}) (Wright 1978) among RSs by the BIOSYS-1 pc program package. Furthermore, we calculated RS-specific F_{IS}-values with this software, to check whether or not possible low genetic variability might result from regional inbreeding. For direct comparison with allozymic variability of brown hares from 20 local samples from Austria (Hartl et al.1993) and eight regional samples from Bulgaria (Suchentrunk et al. 2000), we adjusted all samples to 49 loci by omitting the Acy-1, β-Gal, Ada-2, Ada-3, and Dia-1 loci. All these data sets were produced in our laboratory by using respective marker individuals on the gels, which enabled a direct comparison.

MtDNA-RFLP analysis

We used liver tissue samples of 36 hares (NZ-W = 10, NZ-H = 7, UK-W = 3, UK-L = 13, UK-D = 3) to isolate mtDNA by CsCl/EtBr density gradient ultra-centrifugation and removing EtBr by isoamyl alcohol extraction, basically following the protocols of Lansman et al. (1981) and Rickwood (1987); for details see Hartl et al. (1993) and Nadlinger (1994). We digested total mtDNA of each hare with the following set of 16 hexanucleotid-recognizing type II restriction endonucleases (Roche): ApaI, BamHI, BclI, BglII, ClaI, DraI, EcoRI, EcoRV, HindIII, HpaI, PstI, PvuII, SacI, XbaI, XhoI, and XmnI. The resultant fragments were separated electrophoretically (80 V, two hours) in 0.7% agarose gels with 0.5 µg EtBr/ml and visualized in UV light. In order to validate length estimations of >4 kb long fragments, electrophoresis was continued for two additional hours and fragment measurements were repeated. Fragment length variants <0.4 kb could not be detected by our procedure. Fragment lengths were determined by comparing with Lambda phage DNA digested with HindIII and a 100 bp-ladder (1500–500 bp range). We compared fragments and cleaveage sites deduced from enzyme-specific fragment patterns with those already found in brown hares from Austria (Hartl et al. 1993; Nadlinger 1994). Because our enzyme set was identical with that used already for Austrian brown hares, we could compare our restriction morphs, haplotypes, and indices of mtDNA variability directly with brown hare mtDNA data published by these authors (Hartl et al. 1993). We calculated haplotype diversity (h) and nucleotide diversity (π) (Nei and Li 1979; Nei 1987) to describe RS-specific gene pool variability. We calculated pairwise net nucleotide diversity between RSs based on cleaveage site variations (e. g., Avise 1994) to obtain estimates of mtDNA differentiation among RSs. We compared frequencies of the standard haplotype (i. e., the by far most common type I; Hartl et al. 1993) and other haplotypes (i. e., all others aggregated) between NZ and UK hares by a one-tailed exact Fisher test (hypothesizing lower variability in NZ than UK hares). Finally, we compared RS-specific h- and π-values of NZ and UK hares with the respective values of 18 Austrian brown hare samples (Hartl et al. 1993) by Mann-Whitney U-tests, basing significance decisions on Sequential Bonferroni procedure.

Results

We found di-allelic variation at six loci (Tab. 1) and a significant excess of homozygotes at the Es-1 locus in the NZ-W sample. Except for Acp-1^{50} and Mpi^{84}, all alleles were found earlier in brown hares from central Europe. Allele frequencies did not depend on age category or sex. The indices of genetic variability (H_o, H_e, P, A, H′) are listed in table 1. Overall and locus-specific inbreeding coefficients (F_{IS}) are given in table 2, separately for each RS. Nei's (1978) unbiased D values, Rogers' modified distances, and the fixation coefficients (F_{ST}) appear in table 3, along with the significances of pairwise comparisons of allele frequencies at one or more loci. In table 4, the indices of allozymic variability (based on 49 loci) of the UK and NZ samples are compared to those of 20 Austrian and eight Bulgarian regional samples of brown hares studied earlier (Hartl et al.1993, Suchentrunk et al. 2000).

Thirtyfive (97.2%) hares had the standard mtDNA haplotype I, that was already

Table 1. Allele frequencies at polymorphic loci, sample-specific indices of genetic variability, and mean inbreeding coefficients (F_{IS}) in brown hares from New Zealand (NZ) and Britain (UK); for acronyms see figure 1. Allele designations correspond to those given in HARTL et al. (1989, 1993), SUCHENTRUNK (1993), SUCHENTRUNK et al. (1998), and SUCHENTRUNK et al. (2000). n = number of individuals screened for each locus and regional sample. Indices of gene pool variability (based on 52 loci): H_o = observed average heterozygosity, H_e = expected average heterozygosity, $P_{(99\%)}$ = rate of polymorphism (99% criterion), A = average number of alleles per locus, H′ = Shannon Weaver information index.

Locus	Allele	NZ-W	NZ-H	UK-W	UK-L	UK-D
Idh-2	n	32	28	20	19	20
	100	1.0	1.0	0.975	1.0	1.0
	130	0.0	0.0	0.025	0.0	0.0
Pep-2	n	32	28	20	19	20
	100	0.781	0.536	0.850	1.0	0.875
	104	0.219	0.464	0.150	0.0	0.125
Acp-1	n	32	28	20	19	20
	100	1.0	1.0	1.0	1.0	0.975
	50	0.0	0.0	0.0	0.0	0.025
Ada-2	n	32	6	18	17	18
	100	0.813	0.750	0.694	0.588	0.639
	75	0.187	0.250	0.306	0.412	0.361
Es-1	n	32	28	20	19	20
	−100	0.781	1.0	0.600	0.632	0.650
	−75	0.219	0.0	0.400	0.368	0.350
Mpi	n	32	28	20	19	20
	100	0.938	0.964	1.0	1.0	1.0
	84	0.062	0.036	0.0	0.0	0.0
H_o		0.019	0.013	0.026	0.017	0.023
H_e		0.022	0.019	0.024	0.019	0.023
$P_{(99\%)}$		7.69	5.77	7.69	3.85	7.69
A		1.08	1.06	1.08	1.04	1.08
H′		1.767	1.408	1.829	1.336	1.795

Table 2. Locus-specifc (unbiased) heterozygosities in % (upper values) and inbreeding coefficients (lower values) as well as overall inbreeding coefficients (F_{IS}) for regional samples of brown hares from New Zealand (NZ) and Britain (UK).

	NZ-W	NZ-H	UK-W	UK-L	UK-D
Idh-2	–	–	5.0 −0.026	–	–
Pep-2	34.7 +0.086	50.6 +0.138	26.2 −0.176	–	22.4 −0.143
Acp-1	–	–	–	–	5.0 −0.026
Ada-2	31.0 −0.231	40.7 +0.556	43.7 −0.178	49.9 +0.029	47.5 −0.084
Es-1	34.7 +0.0451	–	49.2 −0.042	47.8 +0.095	46.7 +0.121
Mpi	11.9 −0.067	7.0 −0.067	–	–	–
overall F_{IS}	+0.095	+0.292	−0.117	+0.061	−0.014

found in the majority of central European brown hares (HARTL et al. 1993), and only one hare of the NZ-H sample had a new haplotype. This haplotype deviated from haplotype I by only one additional cleavage site, produced by XbaI at position 14.8 kb of the restriction map published in HARTL, et al. (1993). Values of haplotype (h) and nucleotide diversity (π) were zero for all RSs except for the NZ-H sample; that had an h-value of 28.57% and a π-value of 0.049%. Haplotype frequencies did not differ significantly between the RSs from Britain and New Zealand. Also, RS-specific h- and π-values of the samples from Britain and New Zealand were not significantly lower ($p > 0.05$, one-tailed Mann-Whitney U-tests) than in brown hares from 20 Austrian localities (cf. HARTL et al. 1993). Values of pairwise net nucleotide diversities

Table 3. Matrix of pairwise genetic distances and fixation indices (F_{ST}) among regional samples of brown hares from New Zealand and Britain. NEI's (1978) unbiased D values (first row) and modified ROGERS' distances (WRIGHT 1978) (second row) above the diagonal and F_{ST} values below. F_{ST} values were considered differing significantly from zero with significant allele frequencies for at least one locus in pairwise comparisons (significance based on Sequential Bonferroni procedure; nominal $\alpha = 0.05$, 45 tests). Significance (sig) or no significance (n. s.) is indicated below each F_{ST} value. For acronyms of regional samples see figure 1.

regional samples	(1)	(2)	(3)	(4)	(5)
NZ-W (1)	–	0.001 0.047	0.001 0.033	0.001 0.049	0.001 0.034
NZ-H (2)	0.052 sig	–	0.004 0.071	0.007 0.085	0.004 0.070
UK-W (3)	0.024 n. s.	0.109 sig	–	0.000 0.026	0.000 0.019
UK-L (4)	0.057 sig	0.167 sig	0.016 n. s.	–	0.000 0.019
UK-D (5)	0.026 n. s.	0.106 sig	0.003 n. s.	0.009 n. s.	–

Table 4. Comparison of indices of allozymic and mtDNA variability of regional samples of brown hares from New Zealand, Britain, Austria, and Bulgaria. Allozyme data are based on 49 loci and mtDNA RFLP data on 16 restriction endonucleases (see Material and methods). Means and range (in parentheses) of observed (H_o) and expected (H_e) average heterozygosity, Shannon-Weaver diversity index (H'), rate of polymorphism (P – 99% criterion), average number of alleles per locus (A), haplotype (h) and nucleotide diversity (π) are given for each group of local samples. sig = significance as determined by Mann Whitney tests (d. f. = 1) and sequential Bonferroni procedure ($\alpha = 0.05$), n. s.= not significant.

index	2 NZ and 3 UK regional samples (this study)	20 Austrian (HARTL et al. 1993) and 8 Bulgarian (SUCHENTRUNK et al. 2000) regional samples	sig.
H_o (%)	1.26 (0.9–1.7)	2.83 (1.7–4.2)	< 0.0001
H_e (%)	1.4 (1.0–1.7)	3.06 (2.3–4.7)	< 0.0001
H'	1.029 (0.66–1.29)	2.431 (1.73–3.66)	< 0.0001
$P_{(99\%)}$	4.90 (2.04–6.12)	11.15 (8.16–16.33)	< 0.0001
A	1.048 (1.02–1.06)	1.144 (1.08–1.2)	< 0.0001
H_e/P	0.314 (0.245–0.490)	0.279 (0.172–0.451)	n. s.
h (%)	5.7 (0–28.57)	15.9 (0–69.9)[1]	n. s.
π (%)	0.0098 (0–0.049)	0.0351 (0–0.184)[1]	n. s.

[1] mtDNA values calculated only from data of 18 Austrian regional samples

were zero for the British and NZ-W samples, and amounted to 0.0041% for all pairs involving the NZ-H sample. Respective pairwise values for the presently studied UK and NZ hares and the earlier studied brown hares from 20 Austrian localities (HARTL et al. 1993) ranged between 0.0–0.093%; this was within the range (0.0–0.113%) of net nucleotide diversity between local samples of brown hares from Austria (calculated from data produced in our laboratory, see HARTL et al. 1993).

Discussion

The levels of cross nuclear and mtDNA variability of brown hares from New Zealand and Britain are similar. This is in contradiction to the hypothesis of reduced gene pool variability in hares from New Zealand, owing to an assumed bottleneck during the period of their introduction. Possibly, unrecorded liberations of hares from diverse provenences in Europe in addition to those from the reportedly small number of founders in Australia (LEVER 1985) have increased the effective population size during the founder period in New Zealand. But such additional imports from Europe to New Zealand do not seem very likely, given the acclimatized hares already available in Australia for the Acclimatization Companies.

The essential point to explain the absence of reduced genetic variability in hares from New Zealand as compared to British hares is that brown hares from both New Zealand and Britain exhibit clearly lower allozymic variability than continental European populations. Theoretically, the small number of allozymic alleles found presently in the British brown hares could have been sampled by only few individuals. All common alleles (i. e., those with relative electrophoretic mobility 100/–100) of the British samples were also common in brown hares from diverse regions of continental Europe (HARTL et al. 1989, 1990, 1992, 1993, 1994; SUCHENTRUNK et al. 2000; SUCHENTRUNK et al. unpubl. data). But only 16% of all variant alleles found so far in continental European brown hares with the same set of loci (cf. HARTL et al. 1994; SUCHENTRUNK et al. 2000) occurred in the British samples. Quite several with wide distribution in continental Europe (Pgd^{129}, $Hk\text{-}2^{67}$, $Es\text{-}1^{-42}$, $Es\text{-}l^{-108}$, $Es\text{-}D^{141}$, Mp^{126}) are likely absent in British brown hares. And only one (7.1%) of all mtDNA haplotypes found so far in brown hares from central and southeastern Europe (HARTL et al. 1993, 1994; NADLINGER 1994; SUCHENTRUNK, unpubl. data) could be detected in British brown hares. But quite a number of regional samples of Austrian brown hares did also not display any mtDNA variability (HARTL et al. 1993). British sample sizes are probably too low for such a comparison. Nevertheless, absence of any mtDNA variability of the presently studied brown hares from Britain and the fact that only the standard European mtDNA haplotype I was found in Britain, as opposed to its significantly ($p < 0.0001$, exact Fisher's test) lower frequency on the continent, agrees with the interpretation of generally reduced genetic variability in British brown hares.

The low genetic variability of British brown hares might result from an "ancient" population bottleneck or long-term low effective population sizes associated with their colonization history. It has been hypothesized that brown hares were introduced in Roman times (cf. CORBET 1986; ARNOLD 1993). Deliberate releases of only few individuals or occasional escapes from farms in Roman times or earlier during the Mesolithic (?) or Neolithic occupation periods (cf. JONES and KEEN 1993) could have resulted in a poor gene pool variability of the pioneer population. In addition, a low survival rate owing to a high predation pressure by foxes and other predators, pathogenes, adverse weather conditions etc., could have hampered a quick population growth in the wild. Long-term low effective population size effectively reduces allelic variabililty and heterozygosity (e. g., CHAKRABORTY and NEI 1977; HEDRICK 1985). Contrary to the hypothesis of a deliberate introduction of brown hares to Britain in Roman

times, YALDEN (1982) and ROBERTS (1994) list brown hares along with mountain hares (*Leptus timidus*) as native to Britain since late-glacial times (see also GRIGSON 1983 for the Later Mesolithic excavation site of Cherhill, JONES and KEEN 1993, and citations therein). In this case, brown hares could have lost genetic diversity at low densities under adverse late-glacial climate, or later on in small isolated pockets. STUART (1982), however, lists "*Lepus* sp." as an element of the early Flandrian fauna of Star Charr, and considers only *L. timidus* definitly recorded as fossil in Britain (cf., MAYHEW 1975). As to our knowledge, fossil evidence of *Lepus europaeus* is uncertain in north-central and north-western mainland-Europe during the late-glacial period and early Flandrian, before the formation of the Channel (Strait of Dover, c.8000 years BP cf. e. g., JONES and KEEN 1993). Brown hares might not have roamed mid-latitude Europe during the early Flandrian and not have managed to arrive there before the formation of the Channel (see also CORBET 1986). Alternatively, genetic diversity could have been reduced in fragmented populations with long-term low densities in suboptimal habitats after woodland regeneration in the post-Roman period. But it seems that the distribution of wooded and non-wooded land in Britain has not been altered much in the post-Roman period (ROBERTS 1994).

The low allozymic diversity of British brown hares unlikely results from recent inbreeding in regional populations. This is indicated by quite normal locus-specific heterozygosities, lack of heterozygote deficiencies, and the generally low or even negative inbreeding coefficients. This interpretation is also supported by the absence of a significant gene pool substructuring. Allele frequencies of British brown hares do not differ much across regions. Insignificant fixation indices (F_{ST}) and genetic distance values indicate absence of drift effects among local populations. Obviously, the low amount of genetic variability contained in British brown hares is partitioned among individuals within local populations rather than across larger geographic ranges. The Acp-1^{50} allele occurred exclusively in the Scottish sample and was not found in a large number of brown hares from many regions in continental Europe. It might result from a recent local mutation.

Differentiation between the gene pools of British and central European (Austrian) brown hares (cf., HARTL et al. 1993) is negligible, and virtually nil when based on the detected mtDNA haplotypes. The corresponding pairwise NEI's (1978) unbiased D values (0.000–0.005) fall within the range encountered among local populations from Austria (e. g., HARTL et al. 1993). Despite their conventional subspecific position (*L. e. occidentalis*, DE WINTON, 1898), British brown hares represent only a genetically depauperate version of brown hares from continental Europe. This is confirmed by the absence of any other mtDNA haplotype apart from haplotype I, which has a phylogenetically central position in brown hares and is most widespread in central Europe (HARTL et al. 1993; SUCHENTRUNK, unpubl. data). As indicated by the low genetic distances, no significant genetic drift has occurred between British and continental European brown hare populations. As regards mtDNA, we cannot draw any conclusions on differentiation among the regions studied in Britain, because of too low sample sizes for two regions. Also, the present data do not permit any conclusion as to origins of the British brown hares. This might be achieved by comparing regional population samples and continental European samples with a highly resolving molecular marker system (e. g., microsatellites, mitochondrial d-loop sequences).

The lack of distinct gene pool structuring and the poor allozymic diversity of British brown hares suggest that a relatively high proportion of variant alleles was sampled by the few individuals that supposedly constituted the founder populations in New Zealand. Theoretically, all currently found alleles of the British brown hares could have been contained in the founders.

In New Zealand, hares spread rapidly after their introduction (FLUX 1990). Presently, they occupy large ranges including practi-

cally all types of habitats on the North and South Islands. Although they commonly do not reach those high densities as in Europe, they even have established successful populations in sub-alpine (PARKES 1984) and rather harsh alpine environments (FLUX 1967). Their successful spread in New Zealand might have been particularly favoured by a low level of parasitic or infectuous diseases, a long-term low level of agrochemistry, and relatively little intensification of farming machinery, little road mortality, low predation pressure on adults etc. (see also FLUX 1990). This rapid increase of the founder populations on both main islands, together with repeated imports and subsequent translocations within and between the islands by diverse naturalization companies (FLUX 1990) probably prevented severe loss of genetic variability. Generally, quick population increases following a bottleneck, as was reported for the founder populations of Phillip Island (ROLLS 1969) and New Zealand (FLUX 1990), are counteracting reduction of allelic variability in founder populations (e. g., NEI et al. 1975).

As expected, particularly rare alleles (Idh-2^{130}, Acp-1^{50}) of British brown hares are absent in hares from New Zealand, whereas the common British alleles are common in New Zealand too. Interestingly, the Mpi^{84} allele of both populations from New Zealand was neither present in the British brown hares nor in any of over 900 brown hares from diverse regions of continental Europe studied so far (HARTL et al. 1994; SUCHENTRUNK et al. 2000; SUCHENTRUNK, unpubl. data). It might stem from a recent mutation in New Zealand or Australia. Alternatively, it could be present in regions of Britain or anywhere else in Europe that were not yet sampled, but wherefrom hares were shiped to Australia. The same interpretations apply to the exclusive occurrence of mtDNA haplotype V in the Harpa/Avoca catchment on the South Island of New Zealand. This haplotype is phylogenetically closely related to the basal mtDNA haplotype I of European brown hares, and its evolution can be explained by only one base substitution.

The two local populations on both main islands of New Zealand have differentiated little since their foundations over one hundred years ago. The nuclear and mitochondrial gene pools of hares from New Zealand are very similar to those of British brown hares. Lack of data of allele frequencies in the source population, unknown effective population sizes, and repeated translocations of hares within and between the North and South Islands, that might have changed allele frequencies, prevented us to compare the presently observed allele frequencies with theoretical frequencies as resulting from drift simulations (see e. g., FITZSIMMONS et al. 1997). But both NEI's (1978) D and F_{ST}-values between British and New Zealand populations largely fall within the ranges encountered among regional populations in Europe ($D = 0.000–0.019$, $F_{ST} = 0.00–0.124$, re-calculated from adjusted data sets of 49 loci, cf. HARTL et al. 1993; SUCHENTRUNK et al. 2000). In fact, only the F_{ST}-value for the NZ-H and UK-L samples slightly exceeds the range for regional samples from mainland Europe. This marginally increased level of differentiation between these two populations apparently results from the absence of the Pep-2^{104} allele in UK-L and the Es-1^{-75} allele in NZ-H. But in general, we cannot see any marked reorganization in the cross gene pool of hares from New Zealand as compared to British brown hares. Rapid population increases in the local founder populations, repeated releases, and multiple transfers of hares by naturalization companies (FLUX 1990) likely prevented strong genetic drift. However, in spite of apparently well adapted populations in Britain and New Zealand (SUCHENTRUNK et al. 1998), brown hares from these countries harbour less genetic resources for adaptation to future environments than populations from mainland Europe.

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Zusammenfassung

Geringe Allozym- und mtDNA-Variabilität bei neuseeländischen und britischen Feldhasen (*Lepus europaeus*) – eine Folge von Flaschenhälsen?

Untersucht wurde die Allozym- und mtDNA-Variabilität bei britischen und neuseeländischen Feldhasen (*Lepus europaeus*), um die Hypothese eines Variabilitätsverlustes bei neuseeländischen Hasen infolge eines Flaschenhalsereignisses („Gründer-Effekt") bei ihrer Einbürgerung im 19. Jahrhundert zu überprüfen. Bei 119 Hasen aus drei britischen und zwei neuseeländischen Stichprobengebieten wurde mittels horizontaler Stärkegelelektrophorese ihre allelische Variabilität an 52 Strukturgenloci ermittelt. Bei 36 Hasen wurde anhand von 16 6-Basenschneidenden Endonukleasen der Restriktionsfragmentlängenpolymorphismus (RFLP) in der gesamten mitochondrialen DNA (mtDNA) analysiert. Alle Indices der Allozym- und mtDNA-Variabilität lagen bei den neuseeländischen Stichproben im Bereich jener der britischen Feldhasen. Somit kann die Hypothese zu Verlusten an genetischer Variabilität bei neuseeländischen Hasen im Vergleich zu den britischen nicht aufrecht erhalten werden. Ausgeprägte genetische Drifteffekte zwischen britischen und neuseeländischen Feldhasen konnten ebenfalls nicht festgestellt werden. Jedoch zeigten alle fünf untersuchten regionalen Stichproben signifikant geringere Allozym-Variabilität als Feldhasen vom europäischen Kontinent. Dies läßt die Vermutung eines Verlustes der genetischen Variabilität bei den britischen Feldhasen im Verlaufe ihrer Besiedlungsgeschichte zu. Die nominell als eigene Unterart geführten britischen Feldhasen (*L. e. occidentalis*, De Winton; 1898) erwiesen sich lediglich als genetisch verarmte Varianten der kontinental-europäischen Feldhasen.

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