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On biochemical genetic variability and divergence of the two Hedgehog species *Erinaceus europaeus* and *E. concolor* in central Europe

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Abstract

Allozymic variation within and between the two closely related hedgehog species *Erinaceus europaeus* (n = 45) and *E. concolor* (n = 40) from their central European contact and overlap zones was studied. Horizontal starch gel electrophoresis of 27 isozyme systems encoding for 37 putative loci was employed, using kidney tissue samples. Average heterzygosity (H_e = 0.019–0.02 in *E. europaeus*; H_e = 0.000–0.02 in *E. concolor*) and rates of polymorphism ($P_{99\%}$ = 5.41 in *E. europaeus*; $P_{99\%}$ = 2.7–5.41 in *E. concolor*) of regional samples were low as compared to mammalian standards. Three loci (Aat-1, Aat-2, Gpi) showed obviously alternately fixed (differential diagnostic) alleles between the two species. There was no indication of introgressive hybridization. Despite low levels of intraspecific genetic distances (Nei's unbiased D for *E. europaeus* = 0.003; and for *E. concolor* = 0.000–0.005), significant substructuring of the gene pools of either species was found. Based on the interspecific genetic distance (Nei's unbiased D = 0.087–0.099), the estimated period of cladogenetic separation amounts to 435,000–495,000 years BP. This accords with the hypothesis of the evolution of the two species during the Pleistocene.

Key words: *Erinaceus europaeus*, *Erinaceus concolor*, hedgehogs, allozymes, electrophoresis

Introduction

The two closely related hedgehog species *Erinaceus europaeus* and *E. concolor* show allopatric occurrence over most parts of their distributional ranges (Reeve 1994). In central Europe, however, a zone of overlap exists in Poland, the Czech Republic, Austria, and Italy (Kratochvíl 1966; Rödl 1966; Kratochvíl 1975; Bauer 1976; Pucek and Raczyński 1983; Lapini and Perco 1987; Filippucci and Lapini 1988). F_1- and F_2-hybrids between the two species as well as backcrosses have been produced in captivity (e.g., Herter 1935; Poduschka and Poduschka 1983), and, according to morphological studies, may occasionally occur in the wild (Herter 1934; Holz 1978; Ansorge 1987).

The central European region of sympatric occurrence provides an opportunity to compare the level of interspecific gene pool divergence to the genetic variability of either species within a restricted geographical range. Given there is no substantial introgression, gene pool divergence between the two species in their overlap zone is expected to be clearly greater than between conspecific regional samples, even if the latter are compared between more distant sites (Avise 1975; Nei 1987).

Material and methods

Specimens of the western European hedgehog (*Erinaceus europaeus* L., 1758, n = 45) and the eastern European hedgehog *Erinaceus concolor* Martin, 1838, n = 40) were collected as road kills in the Upper Lusatia (Oberlausitz) region (eastern Germany, see also Ansorge 1987) and in various parts of Austria between June 1987–June 1997. Sampling locations of some of the Austrian specimens were reported in Spitzenberger (1995) and Egermann (1996). Species determination was carried out using morphological criteria (cf., Herter 1934; Kratochvíl 1975; Wolff 1976). There were no problems with species determination of any individual by using morphological criteria (metric and nonmetric skull and mandible characters, head and ventral coat colouration and pattern). Details of sampling localities are given in figure 1. Specimens of *E. europaeus* were grouped into two regional samples: Upper Lusatia (EE-UL, n = 40) and Austria (EE-A, n = 5). Specimens of *E. concolor* were grouped into three regional samples: Austria, north of the river Danube (EC-ND, n = 11), Austria, south of the river Danube but north of the Alps (EC-SD, n = 24), and Austria/Carinthia (Kärnten, south of the main Alpine range) (EC-C, n = 5). Apart from this regional grouping, all specimens found within or at the edge of the Austrian overlap zone (Lower and Upper Austria, n = 15) were considered in a second approach for an inter-specific comparison. Their morphological features did not provide any ambiguity in species determination. Four of these hedgehogs were determined morphologically as *E. europaeus* and 11 as *E. concolor.*

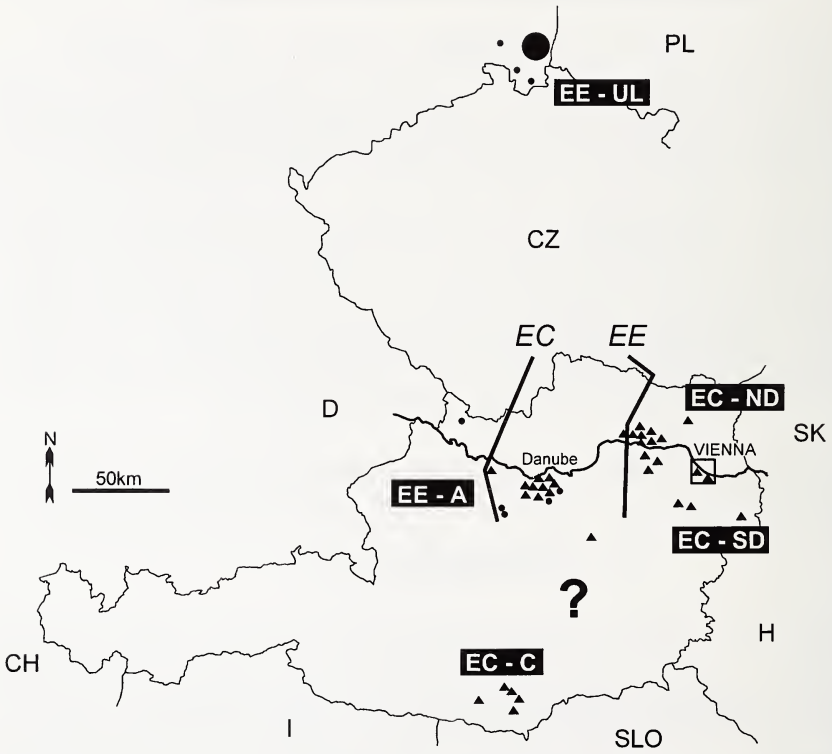

Fig. 1. Sampling localities of western (circles) and eastern (triangles) European hedgehogs in Austria and eastern Germany.
One or more individuals per symbol. The two vertical lines in Austria delineate the eastern edge of the range of the western European hedgehog (EE), and the western edge of the range of the eastern European hedgehog (EC) in the provinces of Upper and Lower Austria (cf. Bauer 1976). The question mark indicates absence of published data on distribution in this part of Austria. Acronyms of regional samples of western European hedgehogs: EE-UL = Upper Lusatia (most from Görlitz and environs), EE-A = Austria; of eastern European hedgehogs: EC-ND = Austria north of the river Danube; EC-SD = south of the river Danube; EC-C = southern Austria, province of Carinthia.

Kidneys of all hedgehogs were stored at –20 °C until processed. Preparation of kindey tissue samples, electrophoresis, and protein-specific staining were performed according to Hartl and Höger (1986) and Grillitsch et al. (1992). Isozyme loci were designated by numbers starting with "1" as the most anodal (cf., e.g. Rothe 1994). For resolving allelic variants migrating allozymes of individuals of both species were compared side-by-side on the same gels. Letters with negative signs denoted cathodal migrating allozymes. Genetic interpretation of electromorphs followed the principles given in Rothe (1994).

The following 27 isozyme systems encoded by 37 presumptive 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): 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), glucose dehydrogenase (GDH, 1.1.1.47, Gdh), glutamate dehydrogenase (GLUD, 1.4.1.3, Glud), NADH-diaphorase (DIA, 1.6.2.2, Dia-1, -2), superoxide dismutase (SOD, 1.15.1.1, Sod-1), purine nucleoside phosphorylase (NP, 2.4.2.1, Np), aspartate aminotransferase (AAT, 2.6.1.1, Aat-1, -2), glutamate pyruvate transaminase (GPT, 2.6.1.2. Gpt), hexokinase (HK, 2.7.1.1, Hk-1), creatine kinase (CK, 2.7.3.2, Ck-2), adenylate kinase (AK, 2.7.4.3, Ak-1, -2), phosphoglucomutase (PGM, 2.7.5.1, Pgm-1), esterases (ES-D, 4.2.1.1, Es-D), acid phosphatase (ACP, 3.1.3.2, Acp-1, -2), fructose-1,6-diphosphatase (FDP, 3.1.3.11, Fdp), β-galactosidase (β-GAL, 3.2.1.23, β-Gal), peptidases (PEP, 3.4.11, Pep-1, -2), aminoacylase (ACY, 3.5.1.14, Acy), adenosine deaminase (ADA, 3.5.4.4, Ada-1), fumarate hydratase (FH, 4.2.1.2, Fh-1, -2), aconitase (ACO, 4.2.1.3, Aco-1), mannose phosphate isomerase (MPI, 5.3.1.8, Mpi), glucose phosphate isomerase (GPI, 5.3.1.9, Gpi).

The BIOSYS-1 pc package (Swofford and Selander 1989) was used 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 (A), deviation of observed genotypes at polymorphic loci from Hardy-Weinberg expectations by calculating exact significance expectations and pooling of genotypes of loci with more than two alleles, F-statistics for estimation of partitioning of relative genetic variability, genetic distances (Nei's (1978) D and Rogers' distances), and to summarize genetic relationships between regional samples by cluster analyses (UPGMA dendrogram and Wagner unrooted tree). Heterogeneity of allele frequencies at polymorphic loci across the respective geographical samples of *E. europaeus* and *E. concolor* was proved by Fisher's exact test and the G-test.

Results

A total of 2750 genes of *E. europaeus* and of 2908 genes of *E. concolor* were analyzed by means of their products (proteins). Allelic variation was observed at six loci (Tab. 1). However, polymorphism was found only at the Mor-2 and the Pep-1 loci in *E. europaeus*, and at the Gpi and the Pep-1 loci in *E. concolor.* At the Aat-1, Aat-2, and Gpt loci alleles were obviously alternately fixed for the two species. Allele frequencies, observed and expected heterozygosity, rate of polymorphism and average number of alleles per locus are listed in table 1, separately for each regional sample. There was no significant deviation of genotype frequencies from Hardy-Weinberg expectations at any polymorphic locus. The frequencies of the Mor-2 alleles varied significantly ($p = 0.026$; d. f. = 1, Fisher's exact test) between the two regional samples of western European hedgehogs. In eastern European hedgehogs the Pep-1 allele frequencies varied significantly ($p < 0.025$, d. f. = 2; G-test) across the three regional samples.

A summary of the non-hierarchical F-statistics is presented in table 2, separately for each species. Values of Wright's (1978) hierarchical F-statistics for estimating the relationship between intra- and interspecific partitioning of the relative genetic variability are given in table 3. The matrices of pairwise Nei's (1978) genetic distances and Rogers' distances are given in table 4. Genetic relationships among all hedgehog samples are depicted in figure 2 by a UPGMA dendrogram based on Nei's (1978) D values and in figure 3 by a Wagner unrooted tree using Rogers' distances and midpoint-rooting of the longest path.

Table 1. Genetic variation in *Erinaceus europaeus* and *E. concolor.* Allele frequencies at variable/polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, rate of polymorphism (99% criterion) (P), and mean number of alleles per locus (A) are given for each regional sample. n = number of hedgehogs. For regional sample acronyms, see Material and methods.

Locus		*E. europaeus*			*E. concolor*	
		UL	A	ND	SD	C
n	allele	40	5	11	24	5
Mor-2	a	0.421	0.800	1.000	1.000	1.000
	b	0.579	0.200	0.000	0.000	0.000
Aat-1	a	1.000	1.000	0.000	0.000	0.000
	b	0.000	0.000	1.000	1.000	1.000
Aat-2	-a	1.000	1.000	0.000	0.000	0.000
	-b	0.000	0.000	1.000	1.000	1.000
Gpt	a	1.000	1.000	0.000	0.000	0.000
	b	0.000	0.000	1.000	1.000	1.000
Pep-1	a	0.857	0.800	0.636	0.587	1.000
	b	0.125	0.200	0.364	0.413	0.000
	c	0.018	0.000	0.000	0.000	0.000
Gpi	-a	1.000	1.000	1.000	0.870	1.000
	-b	0.000	0.000	0.000	0.130	0.000
H_o		0.014	0.011	0.015	0.015	0.000
H_e		0.020	0.019	0.013	0.020	0.000
P (%)		5.410	5.410	2.700	5.410	0.000
A		1.080	1.050	1.030	1.050	0.000

Table 2. Polymorphic loci and WRIGHT's (1978) non-hierarchical F-coefficients for *Erinaceus europaeus* and *E. concolor*

Locus	*E. europaeus*			*E. concolor*		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Mor-2	0.244	0.552	0.407	–	–	–
Pep-1	–0.030	–0.007	0.021	0.012	0.186	0.177
Gpi	–	–	–	0.233	0.303	0.091
mean:	0.090	0.308	0.239	0.054	0.207	0.161

Table 3. variance components and WRIGHT's (1978) hierarchical F-statistics combined across loci for *Erinaceus europaeus* and *E. concolor.*

Comparison (X/Y)	Variance component	F_{XY}
regional sample/species	0.103	0.161
regional sample/total	1.570	0.747
species/total	1.468	0.698

Table 4. Pairwise genetic distances between intraspecific regional or species samples. NEI's (1978) D above and modified Rogers' distances (WRIGHT 1978) below diagonal. For sample acronyms, see Fig. 1.

	(1)	(2)	(3)	(4)	(5)
EE-UL (1)		0.003	0.097	0.099	0.096
EE-A (2)	0.063		0.087	0.088	0.087
EC-ND (3)	0.303	0.288		0.000	0.003
EC-SD (4)	0.304	0.290	0.023		0.005
EC-C (5)	0.301	0.289	0.060	0.071	

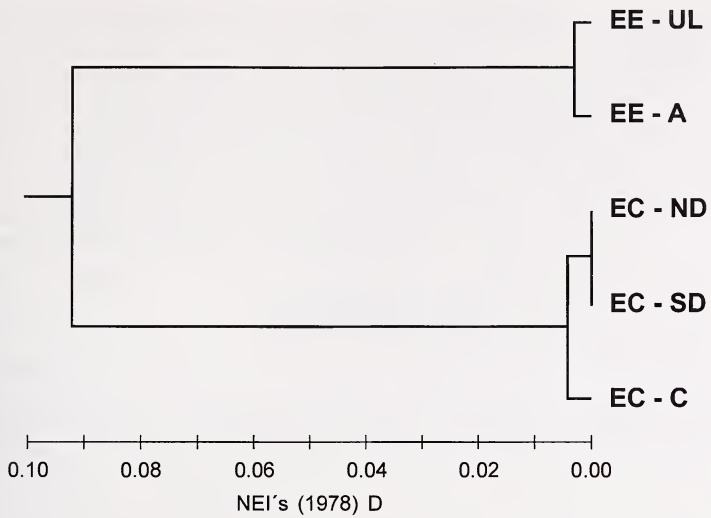

Fig. 2. UPGMA dendrogram depicting genetic relationships among the regional samples of western and eastern European hedgehogs. Cophenetic correlation index = 0.996.

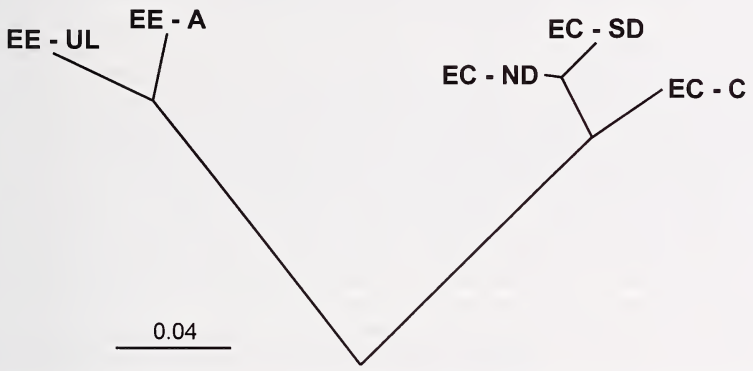

Fig. 3. Wagner dendrogram based on modified Rogers' distances, and rooted by the midpoint of the longest path. Total length of the tree = 0.374; cophenetic correlation coefficient = 1.0.

Regarding the 15 hedgehogs from the overlap zone, there was no indication of introgressive hybridization; species designation by allozymes was consistent with the determination using morphological criteria in each individual. The genetic distances between the two species based on these individuals from the overlap zone (Nei's (1978) D = 0.087; Rogers' distance = 0.289) conformed with the other values of interspecific divergence listed in table 4.

Discussion

Genetic variability within either species was rather low, as compared to mammalian standards (e.g, Nevo et al. 1984; Tolliver et al. 1985; Tiedemann et al. 1996). Nevertheless, the significant variation of allele frequencies at the polymorphic loci in both species indicates some substructuring of their respective gene pools. In *E. europaeus*, the Mor-2^b allele was

currently present only in hedgehogs from north of the river Danube. Among the eastern European hedgehogs, the Pep-1^b allele occurred only in the region south of the river Danube and north of the main Alpine range. These statistically significant findings do not prove the absence of these two genetic variants from outside of the specified regions, but they indicate at least a somewhat reduced gene flow across regions. This is also revealed by the respective fixation indices (F_{ST}); they are within the upper range of values commonly encounterd for genetic partitioning among mammalian populations (cf., Avise 1994). Based on the relationship between the number of migrants (N_m) and the fixation index ($N_m \approx (1 - F_{ST})/4F_{ST}$; Wright 1943), applying to the island model of populations, an average of 0.796 western European hedgehogs per generation exchange their genes between the two regions Upper Lusatia and Austria. The respective value for the eastern European hedgehogs amounts to $N_m \approx 1.303$. The first value is below the level necessary to balance genetic drift between populations, the latter is slightly above (cf., Allendorf 1983).

The genetic distances between the two hedgehog species *Erinaceus europaeus* and *E. concolor*, from portions of their distributional overlap and contact zones in central Europe, are within the range commonly observed among congeneric mammalian species; Nei's (1978) D values are, however, close to the lower limit (e.g., Shotake et al. 1977; Avise and Aquadro 1982; Nei 1987; Filippucci et al. 1991; Janecek et al. 1991; Grillitsch et al. 1992; Rogers and Engstrom 1992; Avise 1994); they are lower than those usually encountered in insectivores, when similar numbers of allozyme loci were studied (e.g., Filippucci et al. 1987; Ruedi et al. 1993; Suchentrunk et al. 1995). Nevertheless, distinct gene pool separation between the two species in the study areas is revealed by most probably, alternately fixed (diagnostic) alleles at three loci (Aat-1, -2, Gpt). The presently found interspecific genetic distances are by far greater (by ca. 20 times for Nei's D, and by ca. 4 times for Rogers' distances) than all those obtained between regional samples of either species. Correspondingly, the overall genetic variability presently encountered in the hedgehogs is mainly partitioned between the two species (69.8%), and to a clearly lesser degree (16.1%) among intraspecific regional samples.

All 15 hedgehogs from the sympatric range could be unambiguously classified as either *E. europeaus* or *E. concolor* by their respective allozyme pattern. In each specimen there was concordance between allozymic and morphological species diagnosis. As already found in the overlap zone in north-eastern Italy (Filippucci and Lapini 1988), there was presently no suspect of introgressive hybridization. However, a much larger sample from the overlap zone has to be studied to exclude the occurrence of occassional hybridization.

The presently encountered level of interspecific genetic differentiation (Nei's (1978) D = 0.087–0.099) is clearly lower than in the Italian section of the species' overlap zone (Nei's (1972) D = 0.212; Filippucci and Lapini 1988). This difference may be due to the different numbers of loci screened, the different tissues used (mainly skeletal muscle in Filippucci and Lapini 1988), or different biochemical conditions of electrophoresis (Nei 1987). Filippucci and Lapini (1988) based their calculations of interspecific genetic divergence on 25 loci. Among these, α-Gpdh, Me (synonymous to Mod, E.C. 1.1.1.40), and Est-3 revealed most probably, alternately fixed alleles between their two species samples; these loci contributed most to interspecific genetic distance. While α-Gpdh was not screened presently, there was no allelic variation at the two Mod loci in our study. Moreover, because of ambiguous electromorphic patterns, we refrained from assigning genotypes at esterase loci in our samples. Hence, the presently found lower genetic distance values may result particularly from the exclusion of these polymorphic loci of Filippucci and Lapini (1988). However, the calculation of genetic distances has been presently based on 37 loci, which is sufficient for reliable estimates of genetic distances, even when gene pool divergence is shallow (Nei 1987).

Dating the cladogenetic event that lead to the two modern hedgehog species *E. europaeus* and *E. concolor*, based on Nei's (1978) D and the average rate of codon substitution ($\alpha = 10^{-7}$, Nei 1975), resulted in a divergence time of 435,000–495,000 years BP. This estimate of speciation time is somewhat below that suggested by Filippucci and Lapini (1988). Nevertheless, both estimates are in good accordance with the (paleontologically unproved) hypothesis of the separation of an ancestral European hedgehog genepool in south-eastern and south-western European refuge areas during the Pleistocene, with subsequent independent evolution and a secondary invasion of both species into central Europe during the Holocene (Herter 1934).

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Zusammenfassung

Zur biochemisch-genetischen Variabilität und Divergenz der beiden Igelarten Erinaceus europaeus und E. concolor in Mitteleuropa

Untersucht wurde die Allozym-Variabilität der Igelarten *Erinaceus europaeus* (n = 45) und *E. concolor* (n = 40) aus ihrem sympatrischen und parapatrischen Vorkommen in Mitteleuropa, sowie ihre genetische Differenzierung mittels horizontaler Stärkegelelektrophorese. Sechs der 37 Strukturgenloci zeigten allelische Variabilität, wobei an drei Loci (Aat-1, Aat-2, Gpi) zwischen den beiden Arten alternativ fixierte (differenzialdiagnostische) Allele vorlagen. Bei jedem Individuum stimmten morphologische und biochemisch-genetische Artdiagnose überein. Es konnte kein Hinweis auf introgressive Hybridisierung festgestellt werden. Trotz generell geringer genetischer Variabilität (*E. europaeus*: H_e = 0,019–0,02; P = 5,41; *E. concolor*: H_e = 0,000–0,02; P = 2,70–5,41), zeigten sich in beiden Arten signifikante regionale Genpool-Unterschiede. Der anhand des interspezifischen genetischen Distanzniveaus (Nei's (1978) D = 0.087–0.099) errechnete theoretische Speziationszeitraum der beiden Arten (vor 435 000–495 000 Jahren) entspricht der Hypothese einer pleistozänen Aufsplitterung eines ursprünglich einheitlichen europäischen Igel-Genpools in ein südöstliches und ein südwestliches Refugialgebiet und anschließender getrennter Evolution zu den beiden heutigen Igelarten.

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