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ORIGIN OF THE YELLOW-BELLIED TOAD POPULATION, *BOMBINA VARIEGATA*, FROM GÖRITZHAIN IN SAXONY

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Analysis of variation at four allozyme loci demonstrated that a population of the yellow-bellied toad in Göritzhain (Germany) does not represent an easternmost relict population, but is descended from Romanian toads introduced there about 15 years ago.

INTRODUCTION

Application of electrophoretic methods to studies of protein variation in natural populations has uncovered unexpectedly high levels of protein variation (Lewontin, 1991). Electromorphs (or their underlying alleles) are rarely uniformly distributed across a species range and often form complex geographic patterns. The study of genetic relatedness of populations together with analysis of geographic patterns can provide cues about the history of the populations (Avise, 1994). Regional differentiation also means that local populations bear "genetic tags" which provide a means of tracing the origin of populations suspected of being introduced.

The European fire-bellied toads, *Bombina bombina* and *B. variegata*, are closely related species with parapatric distributions, which hybridize along their contact zone (Arntzen, 1978; Gollmann, 1984, 1987; Gollmann, Roth & Hödl, 1988; Piálek, 1992; Szymura, 1976, 1988, 1993). Despite hybridization, the species retain their identities and can be easily diagnosed away from a narrow hybrid zone by a variety of morphological, anatomical, behavioural and molecular characters (Szymura & Barton, 1991; Szymura, 1993; Nürnberger *et al.*, 1995; MacCallum *et al.*, 1998). Electrophoretic studies of enzyme variation and mitochondrial DNA showed that both species are differentiated into regional groups (Szymura, 1988). Of the two species, *B. variegata*, which inhabits higher mountains of southern and western Europe as well as the Carpathian Mountains, is more subdivided into geographic groups. Four such groups, corresponding in part to subspecific categories, have been distinguished in *B. variegata*: the Italian group (*B. v. pachypus*), the Balkan group (*B. v. scabra*), the western group and the Carpathian group. The Carpathian group seem to be heterogenous, with south-eastern populations possessing alleles absent over the rest of the range. The western and the Carpathian populations correspond to *B. v. variegata*. The pattern of variation observed in *B. variegata* at allozyme loci is complicated. It involves areas of either high variation or monomorphism, relatively narrow transition zones from one type to the other or broad clinal variation. In any case, the knowledge of geographic variation pattern in *B. variegata* is sufficient to identify the geographic origin of any unknown sample taken from anywhere in Europe with considerable precision.

In this paper I provide information on the origin of *B. variegata* from Göritzhain near Burgstät in Saxony (Fig. 1). The yellow-bellied toads inhabiting this locality are suspected of being introduced. They could be descendants of some 20 toads collected in the Fagaras Mountains in Romania and released in 1978 or 1982 at a site some 500 m from the sandpit in which two permant ponds and several temporary pools, all inhabited by *B.variegata,* are located (Tolke, 1996). An alternative explanation for the presence of *B. variegata* in Göritzhain implies that this population is autochthonous and represents an easternmost relict of a wider distribution of *B. variegata* in the past, in Saxony and Thuringia. Schiemenz (1980, 1981) reported *B. variegata* from the vicinity of Gera, about 50 km west of Göritzhain. Unfortunately, near Gera *B. variegata* have gone extinct (Pontius, 1985).

These two alternative scenarios provide easily testable hypotheses. Depending on the scenario, the yellow-bellied toads from Göritzhain could be genetically similar either to western, or to Romanian toads. A third possibility is that the Göritzhain population might be of mixed descent, and therefore genetically intermediate, with proportions of alleles reflecting relative contribution of the two separate populations.

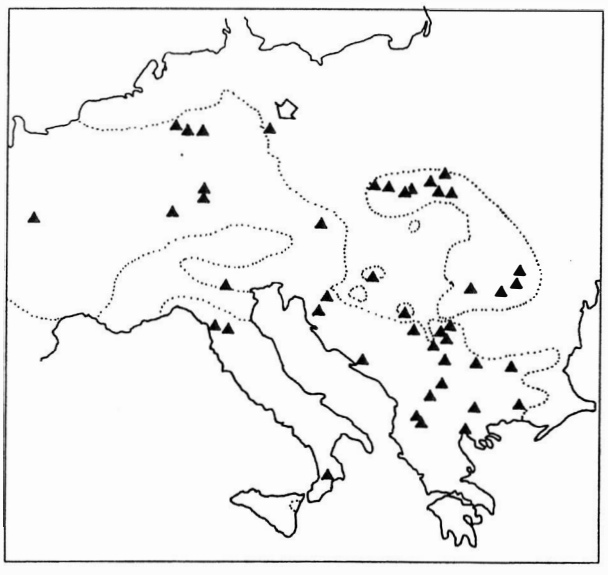

FIG. 1. Location of *B. variegata* samples in Europe. The Göritzhain sample is marked with an arrow. The dotted line indicates the species range.

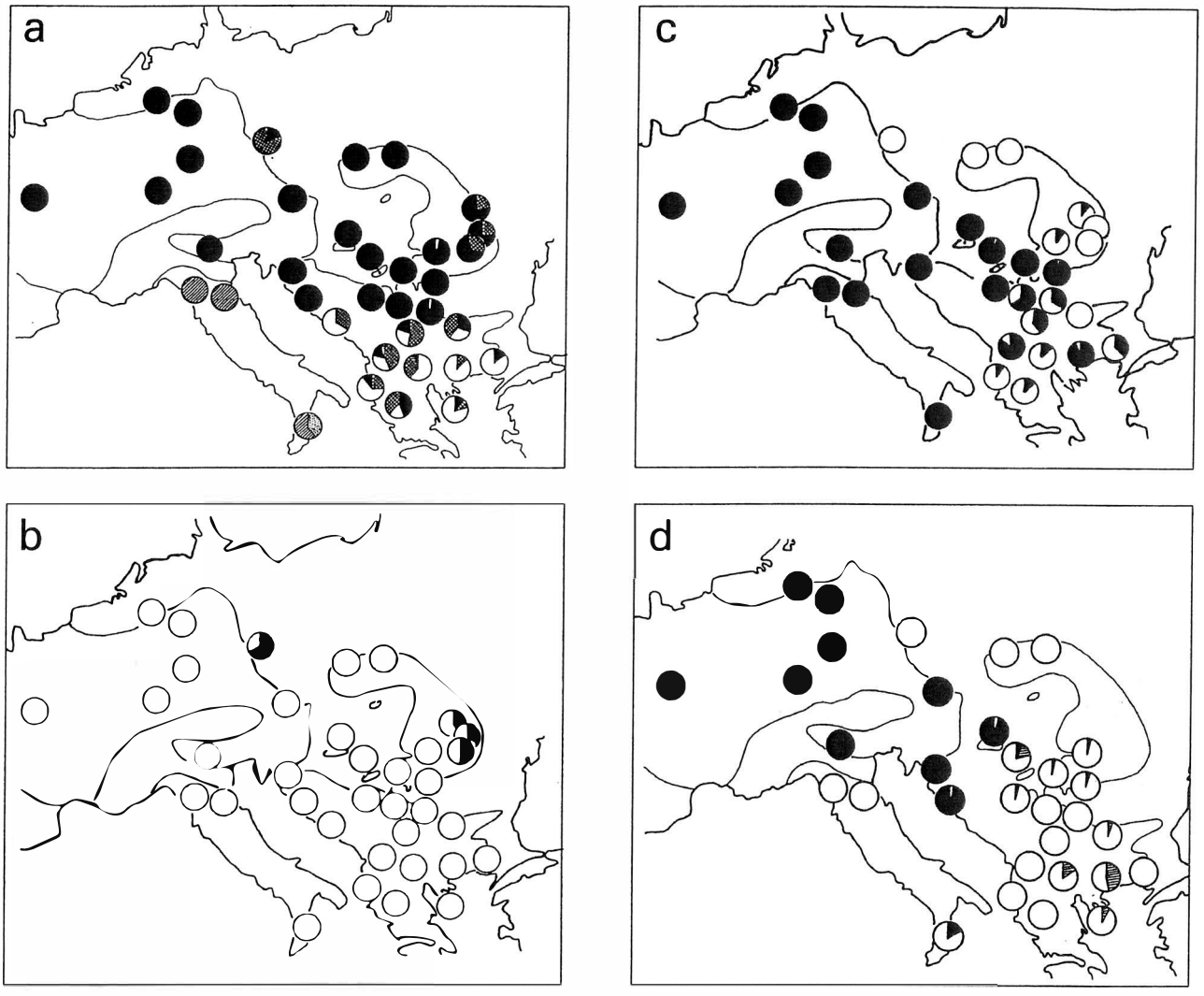

FIG. 2. Geographic patterns of allozyme variation at four enzyme loci in *B. variegata* samples from Europe. (a) Ldh-1: black - Ldh-1 F, crosshatched - Ldh-1 M, white - Ldh-1 R, hatched - Ldh-1 I, stippled - Ldh-1 IR ; (b) Mdh-1: black - Mdh-1 M, white - Mdh-1 F; (c) Idh-1: black - Idh-1 F, white - Idh-1 S; (d) Aat-1: black - Aat-1 S, white - Aat-1 M+F, hatched - Aat-1 R. Thin lines indicate the species boundary.

Allozymes can be studied in extracts prepared from amputated toes (Szymura & Farana, 1978). This method has the advantage of not having destructive effects on a local population, since the animals are released in the place of capture. In addition they are permanently marked and a photograph of their belly pattern provides an easy means of identification in future studies.

METHODS

Nineteen adult *B. variegata* (9 males, 10 females) were captured in June 1994 at the sandpit south of Göritzhain and anaesthetized in 1% solution of MS 222 (Sandoz). The toads were photographed and assigned numbers, and a single middle hind-leg toe was amputated. The toes were frozen on dry ice and later transported to Cracow. All animals recovered from anaesthesia and were released into the ponds.

Toes were homogenized in distilled water, and extracts were subjected to horizontal starch gel electrophoresis (Szymura, 1976, 1983, 1995; Szymura & Farana 1978) along with reference samples from Switzerland, Poland and Romania. These reference samples represent western, Polish and south Carpathian groups of populations. Variation of the following loci was studied: lactate dehydrogenase (Ldh, 2 loci), isocitrate dehydrogenase (Idh, 2 loci), malate dehydrogenase (Mdh, 2 loci) and aspartate aminotransferase (Aat, 2 loci). These loci were chosen, because they represent loci with diagnostic electromorphs (alleles) distinguishing the western populations from the Romanian groups (Szymura, 1988, 1993). Aspartate aminotransferase was studied in a lithium buffer, pH=7.2. Under these conditions, the F-electromorph (Szymura, 1983, 1988) proved heterogeneous and resolved into two forms: Aat-1 F and Aat-1 M. The latter form had slightly greater mobility than Aat-1 S. The Aat-1 F and Aat-1 M are pooled in Fig. 2d, since their distribution in south-eastern Europe is not known.

RESULTS

Of the eight investigated loci four were monomorphic and did not show any variation: Ldh-2,

Mdh-2, Idh-2 and Aat-2. The other four loci, namely Ldh-1, Mdh-1, Idh-1 and Aat-1, showed variation either in the Göritzhain sample or in the reference samples (Fig. 2, Table 1, 2).

The Ldh-1 locus had two electromorphs in the Göritzhain sample, M and F, with respective frequencies of 0.816 and 0.184. The Mdh-1 locus had two alleles, M and F. The M allele predominated; its frequency was 0.737. All individuals were monomorphic for the Idh-1 S allele. The Aat-1 locus was polymorphic with two alleles, M and F, with respective frequencies of 0.222 and 0.778. All three genotypes expected under diallelic variation were observed in the sample.

Genotypic distributions at all three polymorphic loci: Ldh-1, Mdh-1 and Aat-1, conformed to the Hardy-Weinberg equilibrium.

TABLE 1. Genotypes of the Yellow-bellied toads from Göritzhain.

Number	Ldh-1	Mdh-1	Idh-1	Aat-1
1	MF	MF	S	M
2	M	M	S	MF
3	M	MF	S	F
4	MF	MF	S	MF
5	MF	MF	S	MF
6	M	MF	S	F
7	M	M	S	MF
8	MF	MF	S	F
9	M	MF	S	F
10	M	M	S	F
11	M	M	S	F
12	M	M	S	F
13	M	MF	S	F
14	MF	M	S	MF
15	M	MF	S	F
16	M	M	S	F
17	MF	M	S	F
18	MF	M	S	MF
19	M	MF	S	-

DISCUSSION

The western group of *B. variegata* is characterized by the following combination of electromorphs: Ldh-1 F, Mdh-1 F, Idh-1 F and Aat-1 S, and thus the Göritzhain population was expected to have the same combination of alleles under the hypothesis of an autochthonous origin (Fig. 2). This hypothesis has to be rejected, since the sample had a different genetic composition. Ldh-1 had two alleles, M and F; Mdh-1 also had two alleles, M and F; Idh-1 was monomorphic with the S allele; and Aat-1 had 2 alleles, M and F. All four loci provide strong and consistent evidence against the null hypothesis.

Suppose the Göritzhain population is of mixed ancestry and foreign alleles were incorporated following mating between local and released yellow-bellied toads from some unspecified population in 1978 or 1982, i.e. 12-16 years prior to the sampling date. If this is the case, the presence of Ldh-1 M and Mdh-1 M alleles indicate an enriched local gene pool. These alleles have, however, higher frequencies than the supposedly local Ldh-1 F and Mdh-1 F alleles (Table 2), so considerable replacement must have taken place. The same hypothesis would also imply that local alleles at the other two loci, Idh-1 F and Aat-1 S, were either completely replaced with foreign Idh-1 S and Aat-1 M/F or their frequencies reduced below detectable frequency 1/38 = 0.026.

Let us consider the time scale required for this proposition. Generation time in *B.variegata* is not

TABLE 2. Allele frequencies at Ldh-1, Mdh-1, Idh-1 and Aat-1 in the sample from Göritzhain, and in the reference samples from Romania (Tinovul Mohos, Predeal, Bilea Valea), Western Europe and Poland. Abbreviations: *n* - number of individuals, NS - not studied. 1, samples pooled from seven localities (cf. Szymura, 1988). 2, samples from the Western Carpathians, seven localities (Szymura, 1988).

		Göritzhain	Romania			W.Europe[1]	Poland[2]
Locus		*n*=19	T.Mohos *n*=15	Predeal *n*=12	B.Valea *n*=23	*n*=90	*n*=245
Ldh-1	M	0.816	0.333	0.375	0.084	-	-
	F	0.184	0.667	0.625	0.916	1.000	1.000
Mdh-1	M	0.737	0.467	0.500	-	-	-
	F	0.263	0.533	0.500	1.000	1.000	1.000
Idh-1	S	1.000	0.900	1.000	0.870	-	1.000
	F	-	0.100	-	0.130	1.000	-
Aat-1	S	-			0.02	1.000	-
	M	0.222	NS	NS	-	-	0.562
	F	0.778			0.98	-	0.438

known with precision, but it is likely that 3 years (two hibernations) is the shortest this could be. Assuming that the introduction took place 16 years ago and *B. variegata* successfully mated in the same year, five generations of toads have been produced since then. All the studied *B. variegata* were fully mature individuals, and many of them were very large, at least 3 years old and more likely to be older than 5 years. Allele frequency substitution must therefore have taken place in a shorter time, in four or fewer generations. This is highly unlikely, unless the local autochthonous population was very small, say just a few individuals. It is believed that about 20 individuals were released in Göritzhain (cf. Tolke, 1996). If the local population consisted of only 2, 3, 4, or 5 toads, and they mated randomly with the newcomers, allele frequencies typical of the western group at either Idh-1 or Aat-1 locus in the next generation would be 0.091, 0.130, 0.167 and 0.200 respectively. Note that even such a small population consisting of three individuals only, a hardly viable and sustainable population, would transmit a sizeable proportion of the local alleles to the next generation. The local alleles would be difficult to replace within four generations if the mating was random. Complete replacement of local alleles would have to take place independently at Idh-1 and Aat-1 loci, along with an increase of alien M alleles at two other loci, Ldh-1 and Mdh-1. This is a highly unlikely event given independent assortment of the loci (Szymura & Farana 1978, Szymura, 1995). Therefore the hypothesis suggesting mixed ancestry of the Göritzhain *B. variegata* has to be rejected.

The source of the population therefore seems to be Romania. Even without prior knowledge of a possible source for the Göritzhain *B. variegata*, the sample's origin could be located from knowledge of the variation pattern at Ldh-1 and Mdh-1 loci (Fig. 2). The Ldh-1 M allele is present in the Balkans and eastern Carpathians only. Mdh-1 M is a very localized variant observed in the southeastern Carpathian Mountains only. It is this allele which provides a decisive clue to the source of introduced *B. variegata*. The results of the genetic analysis are consistent with the expectation that the Fagaras Mountains was the area in which the ancestors of the yellow-bellied toads inhabiting Göritzhain today were collected.

CONCLUSIONS

Electrophoretic analysis of variation at four enzyme loci demonstrates beyond any doubt that the yellow-bellied toads found in Göritzhain are descended from introduced Romanian *B. variegata*. Although this population may not deserve special protection status under the present law, it offers a unique opportunity to study dispersal and colonization of unoccupied territory. The area could thus offer an unintentional experiment to study population dynamics of species in a modified habitat subject to high human pressure, an environment in which most amphibian species either live or will have to live in the future. The Göritzhain population possesses several enzyme polymorphisms which could help to assess the impact of inbreeding on the loss of genetic variation.

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