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PARTITIONING OF GENETIC (RAPD) VARIABILITY AMONG SEXES AND POPULATIONS OF THE BARN OWL (*TYTO ALBA*) IN EUROPE

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ABSTRACT.—The white Barn Owl subspecies (*Tyto alba alba*) is found in southern Europe and the reddish-brown subspecies (*T. a. guttata*) in northern and eastern Europe. In central Europe, the two subspecies interbreed producing a large range of phenotypic variants. Because of the different ratios of the subspecies in different geographic regions, we predict that genetic variation should be greater in Switzerland than in Hungary. We tested this hypothesis by measuring genetic variation with the RAPD method. As predicted, the genetic differentiation within a Swiss population of Barn Owls was significantly greater than the variation within a Hungarian population. This suggests that gene flow is greater in central Europe than at the eastern limit of the Barn Owl distribution in Hungary. In both countries genetic variation was more pronounced in females than in males. As in other birds, this is probably because female Barn Owls are less philopatric than males. The number of migrants between Hungary and Switzerland is ca. 1 individual per generation; if calculated separately for the sexes, then 0.525 for males and ca. 1 for females (Nm values). The difference in the number of migrants between genders again is likely a consequence of higher male philopatry. The sexual differentiation is greater in the Swiss population than in the Hungarian and the genetic substructuring of the populations of the species is substantial. The reason for the considerable population substructuring could be the nonmigratory behavior and socially monogamous pairing of the species, as well as the geographical barriers (Alps) between the populations examined.

KEY WORDS: *Barn Owl*; Tyto alba; *genetic variability; introgression*; *RAPD*; *subspecies.*

VARIABILIDAD GENÉTICA ESTIMADA MEDIANTE RAPD ENTRE SEXOS Y POBLACIONES DE *TYTO ALBA* EN EUROPA

RESUMEN.—La subespecie *Tyto alba alba* se encuentra en el sur de Europa, y *T. a. guttata* en el norte y oriente de este continente. En el centro de Europa, las dos subespecies se entrecruzan y producen una amplia gama de variantes fenotípicas. Debido a la variación geográfica en la proporción de individuos pertenecientes a las distintas subespecies, predijimos que la variabilidad genética debería ser mayor en Suiza que en Hungría. Pusimos a prueba esta hipótesis midiendo la diferenciación genética usando el

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método de ADN polimórfico amplificado aleatoriamente (RAPD, por sus siglas en inglés). Como lo habíamos predicho, la diferenciación genética dentro de una población suiza de *T. alba* fue significativamente mayor que la variación observada dentro de una población húngara. Esto sugiere que el flujo genético es mayor en el centro de Europa que en el límite oriental de la distribución de *T. alba* en Hungría. En ambos países, la variación genética fue más pronunciada en las hembras que en los machos. Como en otras aves, esto probablemente se debe a que las hembras son menos filopátricas que los machos. El número de migrantes entre Hungría y Suiza es cercano a un individuo por generación, y los valores de Nm calculados por separado para machos y hembras son de 0.525 y aproximadamente 1, respectivamente. Es probable que la diferencia en el número de migrantes se deba nuevamente a la mayor filopatría de los machos. La diferenciación sexual es más pronunciada en la población suiza que en la húngara, y estas poblaciones están sustancialmente subestructuradas genéticamente. Posibles razones para explicar la considerable subestructuración podrían ser el comportamiento no migratorio y el apareamiento monógamo de la especie, además de la separación de las poblaciones estudiadas por barreras geográficas, como los Alpes.

[Traducción del equipo editorial]

In a number of birds, color polymorphism has evolved in allopatry. Under this scenario, a geographical barrier has separated a population in two, a process that facilitates genetic differentiation as gene flow is physically reduced. In some cases, during the time of allopatric separation, stochastic processes or natural selection promoted the evolution of alternative color morphs for which the expression is under genetic control and not sensitive to the environment. After the geographical barrier was removed, the two subpopulations could mix in a zone of secondary contact. If hybrids are viable, color polymorphism allows researchers to trace back the origin of individuals. For example, in the Barn Owl (*Tyto alba*) the white subspecies, *T. a. alba,* is found mainly in southern Europe, whereas the reddish-brown subspecies, *T. a. guttata,* occupies northern and eastern Europe. In central Europe, the two subspecies seemingly pair randomly with respect to coloration (Baudvin 1975, Roulin 1999, Mátics et al. 2002), which implies that hybrids may not be selected against.

Following Voous (1950), these two subspecies may have evolved because during the last ice age, Barn Owls subsisted in two refugia located in southwestern Europe (*T. a. alba*) and southeastern Europe (*T. a. guttata*). After the ice retreated, the two subspecies invaded Europe via Spain and France (*T. a. alba*) and the Balkans (*T. a. guttata*) to meet in a zone of secondary contact in central Europe. The existence of a color polymorphism is consistent with the hypothesis that Barn Owl populations located in central Europe involve two subspecies. From a genetic point of view, we expect that genetic variation should be more pronounced in the zone of hybridization in central Europe than to the east or west. The various extant populations show differential degrees of introgression suggested by the phenotypic distribution of individuals (Mátics et al. 2002, Roulin et al. 2001). In this case, one would expect disequilibria of neutral markers but this was not tested with our methodology. The transition zone appears to be very wide in comparison with other bird species, ranging from west-central France to eastern Hungary and northeastern Poland. On the eastern side of the zone, the reddish-brown form is more frequent comprising 84–92% of the individuals in Hungary (Mátics and Hoffmann 2002) and 90% in southeastern Germany (Schönfeld 1974), whereas the white subspecies prevails on the western side of the zone (Glutz von Blotzheim and Bauer 1980; e.g., 75% in central France [Baudvin 1975] and 50% in Switzerland [Roulin et al. 2001]). The closer the distribution of the subspecies is to the 1:1 ratio in a population the greater variability of the population due to the differences in genetic constitution of the subspecies (Harrison 1993, Arnold 1997). In addition, because the species is socially monogamous and nonmigratory, we predict a relatively high genetic substructuring of the populations. Because in this species, as in other birds (Greenwood 1980), males are more philopatric than females, we expect that in both Swiss and Hungarian populations females are genetically more diverse than males as shown in the Black-billed Magpies (*Pica hudsonia*; Wang and Trost 2001).

We tested these two predictions by quantifying genetic variation using random amplified polymorphic DNA (RAPD) technique. This technique is based on the amplification of unknown DNA sequences using single, short, random oligonucleo-

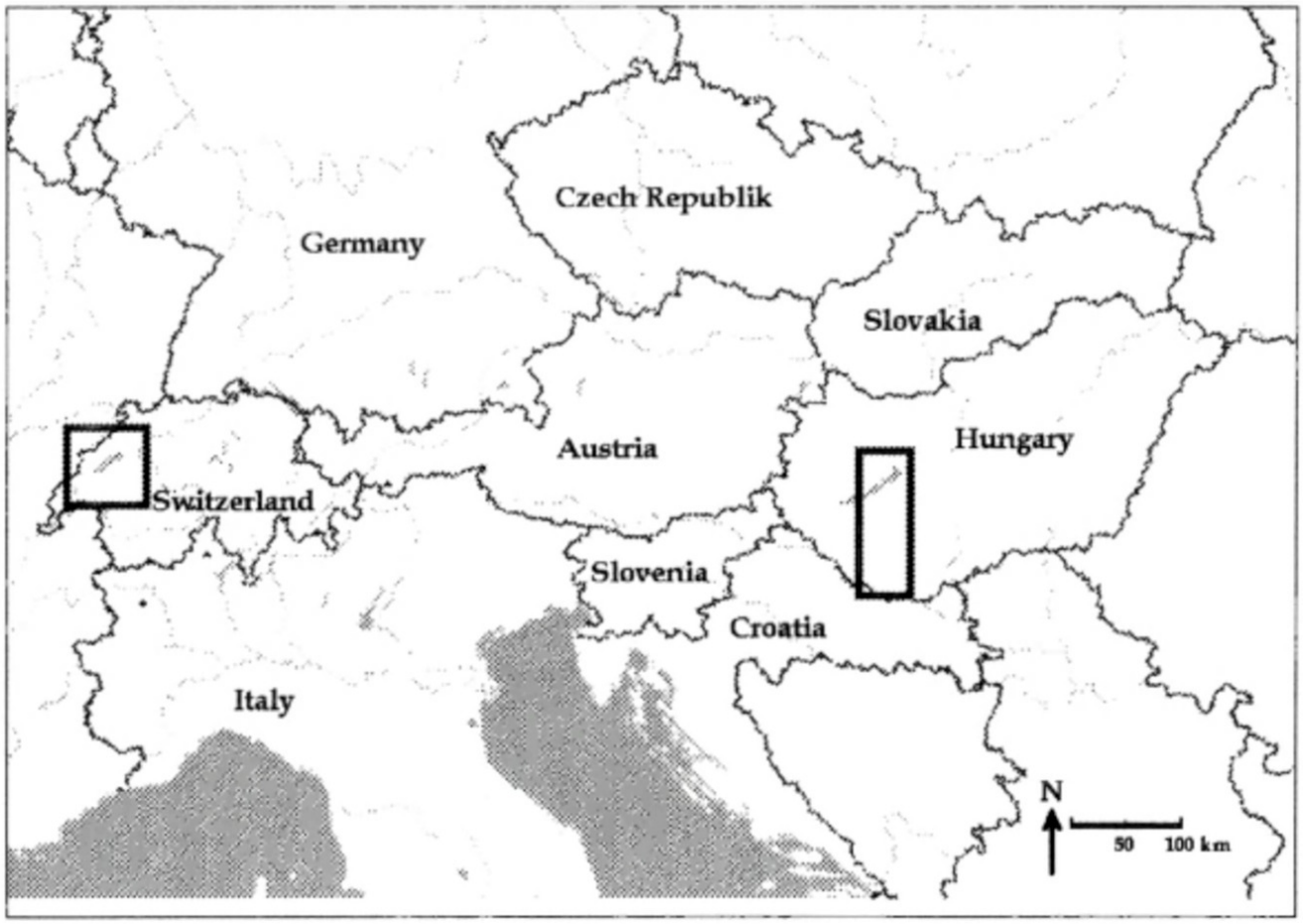

Figure 1. Location of the Barn Owl populations examined for genetic variability. The BIOTA© Association (Pécs, Hungary) provided the base map for this figure.

tide primers. RAPDs provide an unbiased sample of DNA variation along the entire genome (Hwang et al. 2001) including non-nuclear genomes. The sensitivity of RAPDs to population divergence may be derived from rapid evolution of non-coding, repetitive DNA sequences detected by RAPDs (Plomion et al. 1995). Therefore, the RAPD technique is able to detect variation within and among wild populations (Haig et al. 1994, Horn et al. 1996) and among sexes within a population (Wang and Trost 2001). In birds, mainly species with insular distribution (Zwartjes 1999) or living in fragmented habitats (Bouzat 2001) have been analyzed. However, this technique has also been used for other purposes, including sex determination (Park et al. 1997), analysis of wild versus captively-reared populations (Bagliacca et al. 1997), and detection and eradication of hybrids (Negro et al. 2001). Information on the amount of genetic variation within a species and its distribution within and between populations aids conservation planning as well (Hwang et al. 2001).

STUDY AREA

The study areas were located in Hungary (between 47°02′N, 17°33′E and 45°50′N, 18°29′E) and Switzerland (between 46°56′N, 7°03′E and 46°44′N, 6°41′E; Fig. 1). The Swiss study area is located in the middle of the European distribution of Barn Owls and has an elevation between 400–650 m above sea level (masl); the Hungarian study area is near the distribution limit of the species with an elevation between 50–500 masl.

METHODS

Sample Collections. Blood samples from breeding Barn Owls were collected between May and August 1998. First, the skin was cleaned with ethanol over the point where the brachial vein crossed the elbow and insulin needles (diameter = 0.4 mm) were used to collect 100–μL blood. Samples were stored on ice in sterile 1.5 mL Eppendorf tubes until they were carried to the laboratory and frozen at −20°C. The birds were held for at least 5 min after blood collection and then were released.

Table 1. Values of Nei-Li genetic distance among individuals within the groups given. Mean, SE, and Confidence Intervals (CT) are estimated by jackknife procedure (H = Hungary, CH = Switzerland).

	MEAN ± SE	95% CI	99% CI	*N*
H females	0.358 ± 0.006	0.346–0.370	0.342–0.374	13
H males	0.261 ± 0.010	0.241–0.280	0.235–0.287	7
CH females	0.501 ± 0.021	0.460–0.541	0.447–0.554	17
CH males	0.368 ± 0.010	0.349–0.387	0.342–0.394	15
H pooled	0.338 ± 0.004	0.331–0.344	0.329–0.347	20
CH pooled	0.471 ± 0.008	0.455–0.487	0.451–0.492	32

DNA Extraction. Standard phenol-chlorophorm-isoamyl alcohol methods (Sambrook et al. 1989) were used to isolate total DNA from blood samples. Fifty μL-blood samples were suspended in 200-μL PBS buffer and cells were sedimented. The resulting pellet was suspended in extraction buffer containing 20 μg/mL RNAse and was incubated at 37°C for 1 hr. Proteinase K treatment was then applied (to a final concentration of 100 μg/mL) and incubated at 50°C for 3 hr. Samples were extracted three times with equal volumes of phenol followed by ethanol precipitation. DNA was washed with 70% ethanol and resuspended in 100–200 μL sterile-distilled water. The concentration of the extracted samples was quantified using the photometric device GeneQuant (Pharmacia, Cambridge, U.K.).

RAPD PCR Procedure. To find the optimal concentration, where the reactions gave the most consistent and clearest products, DNA was diluted in the range of 10–80 ng/μL (by doubling dilutions). In standard experiments 20-ng DNA in 25 μL reaction volume was used in the presence of 1.5 mM $MgCl_2$. Amplifications were carried out using PTC-150 Minicycler (MJ Research, San Francisco, CA U.S.A.) with the following program: after a first cycle (2.5 min at 94°C, 1 min at 35°C, and 2 min at 72°C), additional 35 cycles were done (40 sec at 94°C, 40 sec at 35°C, and 1 min at 72°C). The products were run on 2% agarose gels. Gels were photographed using the digital-gel documentation system BioDocIt (UVP, Cambridge, U.K.). Twenty-one different primers were tested (QUIAGEN, Budapest, Hungary) and those giving the most variable patterns were used for further analyses (OPW-17, OPW-08, OPT-20, OPN-05, OPO-05, OPH-14, and OPJ-12).

Scoring and Data Analysis. Fragments were visualized by adding 0.1 μg/mL ethidium bromide to the agarose gels. Gel photographs were scored for the presence or absence of RAPD bands. A pair-wise matrix of the genetic distances between individuals was obtained using a euclidean distance measure (Huff et al. 1993), calculated from presence or absence data using RAPDistance (Armstrong et al. 1994). Components of variance partitioned into within- and between-populations were estimated from this matrix using AMOVA program version 1.55 (Analysis of Molecular Variance; Excoffier et al. 1992). The number of permutations for significance testing was set at 1000. Where Φ_{ST} values differed significantly from zero, the number of migrants per generation was calculated using $Nm = 0.25(\Phi_{ST}^{-1}-1)$ (Wright 1951). AMOVA variance components were used as estimates of the genetic diversity within and between populations. Because the genetic distance values were not independent data points, we calculated Nei-Li (Nei and Li 1979) genetic distances as well, followed by jackknife analysis (Shao and Tu 1995), to find differences between populations and genders.

The RAPD technique has some limitations. The most important might be that the banding pattern produced represents nuclear (both sex-linked and autosomal) and mitochondrial genomes. The fact that mitochondrial genes are exclusively maternally inherited, whereas autosomal ones are biparental markers, may obscure interpretation of results, particularly in connection with sexual differentiation and consequences of sex-biased dispersal (Goudet et al. 2002, Prugnolle and de Meeus 2002, Vitalis 2002).

RESULTS

The amplifications with seven primers produced 106 reproducible bands in both populations (ξ = 15.1 bands per primer). In the Hungarian population, 80 of these bands were present and five of them were invariant, whereas in the Swiss samples, 104 were present and no invariant band was found. There were six singletons (bands with one incident; i.e., occurring only in one individual).

The 99% confidence intervals of the two sexes did not overlap. In both populations the jackknife estimates of mean Nei-Li genetic distances of females were greater than those of males (Table 1). The Swiss population showed higher jackknife estimate of mean genetic distance than the Hungarian population because there was no overlap in the 99% confidence intervals (Table 1).

Because the probability that random distance (Φ_{ST}) was greater than the observed distance was less than 0.05 in all cases, the Φ_{ST}-values were significantly different from zero. The AMOVA indicates 7% of the variance is partitioned between the genders within the pooled sample. The Hungarian sample gave similar results (7.2%), whereas the Swiss population was of higher value (14%; Table 2). The among-countries differentiation reaches

Table 2. Results of the analysis of molecular variance with a between-gender and among-countries arrangement.

	df	SS	MS	VARIANCE COMPONENT	Φ_{ST}	P^a
Hungary						
Between genders	1	21.15	21.15	00.966 (7.2%)	0.07	<0.001
Among individuals/within genders	18	222.55	12.36	12.364 (92.8%)		
Switzerland						
Between genders	1	57.91	57.91	2.637 (14%)	0.14	<0.001
Among individuals/within genders	30	476.28	15.88	15.876 (86%)		
Pooled (all females vs. all males)						
Between gender	1	49.30	49.30	1.278 (7%)	0.07	<0.001
Among individuals/within gender	50	843.50	16.87	6.870 (93%)		
Pooled (all Hungarian vs. all Swiss)						
Among countries	1	114.92	114.92	4.04 (21%)	0.21	<0.001
Among individuals/within countries	50	777.89	15.56	15.56 (79%)		

[a] Nonparametric randomization test with 1000 permutations.

21% (Table 2) and the estimated number of migrant individuals between the Swiss and Hungarian populations is 0.96 per generation. The pair-wise Φ_{ST} values gave the same results as the between-sexes arrangement (Table 3). Moreover, the among-males (0.32) and the among-females values (0.20) suggest a higher genetic difference of males between populations. From these data we estimated that 0.53 males and 1.01 females migrate between populations each generation (Nm values).

DISCUSSION

Genetic differentiation among breeding females was greater than among breeding males in both Hungary and Switzerland. The differential dispersal distances of the sexes, males being more philopatric than females, may explain this differentiation. Therefore, males are genetically more similar to each other within a population. This scenario, based on sex-specific dispersal, may also explain the greater among-males differentiation between populations (about twice as many females as males appear to emigrate). Because males do not emigrate as much as females, they preserve the genetic features of their population to a greater degree. This is further supported by the fact that the estimate of migrants per generation (based on genetic data) gave the same result as the ringing data: greater male philopatry (Taylor 1994). Previous studies suggested that, from Hungary, a greater percentage of individuals move toward central Europe than in the opposite direction (Mátics 2003); i.e., the Hungarian population was more of a "source" than a "sink" population. As a consequence, gender- and population-differentiation is greater in Switzerland than in Hungary, because the exchange of individuals is *guttata*- and female-biased. These results were concordant with the fact that both phenotypic and genotypic variability of individuals were greater in the middle of a transition zone than on the edges (Arnold 1997, Roulin 2003). This proposal could be tested with data from other localities such as from western European Barn Owl populations.

Table 3. Pairwise genetic distances of the populations (Φ_{ST} between pairs of populations). Above diagonal is the probability that random distance (Φ_{ST}) > observed distance; (number of iterations = 1000; H = Hungary, CH = Switzerland).

	CH MALE	CH FEMALE	H MALE	H FEMALE
CH male	—	0	0	0
CH female	0.14	—	0	0
H male	0.32	0.29	—	0
H female	0.25	0.20	0.07	—

The between-gender differentiation of the species detected using RAPDs (7%, 14%) seem to be disproportionally high. Using the random priming technique the sexual differentiation was expected to be between 1–2%, as the Barn Owl has 92 chro-

mosomes (Belterman and De Boer 1984). This higher value of gender differentiation could be due to the relatively large size of sexual chromosomes in birds (Stevens 1991). The sexual differentiation of autosomal markers caused by sex-biased dispersal is instantaneous because the next generation receives a random set of alleles from both parents. On the other hand, sex-chromosomal markers preserve the differentiation for longer time and when gene flow occurs continuously, this differentiation could be detected permanently. Differentiation detected in this study therefore may be associated with markers located on sex-chromosomes.

Although only two populations were analyzed, the Φ_{ST} value of 0.21 indicated a substantial genetic substructuring among our study populations. The results of another RAPD study gave Φ_{ST} values of 0.048 and 0.103 for island species (two populations of both the Puerto Rican Vireo [*Vireo latimeri*] and Jamaican Vireo [*V. modestus*], respectively), and 0.015 for a migratory continental species (three populations of the White-eyed Vireo [*V. griseus*]; Zwartjes 1999). For the Greater Rhea (*Rhea americana*) a Φ_{ST} value of 0.0637 was found among four wild and a captive population (Bouzat 2001), which is a low value given that this species is flightless. The special mating system of the Greater Rhea could play an important role in producing this low genetic substructuring. The male rhea establishes a territory and builds a nest. He will then attract a group of about three to six females with whom he mates and they lay ca. 20–30 eggs in his nest. While the females go off to mate with other males, the male will incubate the eggs and look after the chicks on his own. The relatively strong substructuring of the Barn Owl populations could be explained by at least three factors: (1) nonmigratory behaviour, (2) the socially monogamous mating system of the species, and (3) the presence of a geographic barrier (Alps) between the populations analyzed. The Nm value of roughly one is the minimum amount of gene flow that prevents differentiation at neutral loci among populations by genetic drift (Wright 1931).

The conversion of F_{ST}-related values into Nm is problematic for several reasons, including that it is based on isolation-by–distance models. In this study, we could not test for correlations between genetic and geographic distance. Furthermore, population size and dispersal rates are not constant over time and space and assumptions of demographic and genetic equilibrium and uniformity are unrealistic (Whitlock and McCauley 1999). Many assumptions of the models used are violated, so that results cannot be interpreted as direct measures. In addition, other evolutionary forces contribute in establishing differentiation (Bossart and Prowell 1998). Finally, we suggest that drift should play an important role in the microevolution of the Barn Owl as this species of tropical origin (Voous 1988) goes frequently through bottlenecks in the suboptimal European area in hard winters (Taylor 1994).

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