Concordant change in mitochondrial and nuclear genes in a hybrid zone between two frog species (genus *Bombina*)

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Summary. In contrast to results in other studies, nuclear and mitochondrial genes were found to change concordantly in a transect across the hybrid zone between *Bombina bombina* and *Bombina variegata*. mtDNAs of both species are found in populations in the central part of the zone, whereas populations at its margins contain mtDNA corresponding to nuclear genomes.
Key words. Hybrid zone; mtDNA; *Bombina*; enzyme electrophoresis.

In contrast to nuclear genes, mtDNA in animals is passed onto progeny by the female parent alone[2,3]. The fate of mtDNA in populations is therefore governed by rules different from those applying to classical Mendelian units encoded in the nucleus. Several studies have provided evidence for interspecific mtDNA transfer in the absence of nuclear gene introgression[4–6]. Conditions suitable for interspecific transfer of mtDNA may occasionally occur either when sympatric but ecologically separated species interbreed locally or when species with parapatric distributions hybridize along their zone of contact, as do two species of fire-bellied frogs, *Bombina bombina* and *B. variegata*[7–13].

Although no mating preferences are evident in hybrid populations of *Bombina*[12,13], initial hybridization of the two species probably took place between *B. bombina* females and males of *B. variegata*. In the laboratory, amplexus between female *B. bombina* and male *B. variegata* is readily obtained[14], whereas the reverse combination often requires a gonadotropin injection to stimulate the male. In the field, male *B. variegata*, an explosive breeder, is less selective about its mates than *B. bombina*, which has a complex territorial breeding behavior[15,16]. Given these mating preferences in the parental species, F1 hybrids would probably have maternally inherited bombina mtDNA. Such initial matings might also produce 2–3 times as many progeny as the reciprocal matings because *B. bombina* females produce 2–3 times as many eggs per clutch as do *B. variegata* females. Conditions thus appear suitable for interspecies transfer of bombina mtDNA into *B. variegata*.

To investigate this possibility, we analyzed both mtDNAs and nuclear genes of individuals from various locations in a transect across the hybrid zone near Kraków, Poland (fig. 1). Details of mtDNA preparation, restriction enzyme digestion, ^{32}P labeling and gel electrophoresis are described elsewhere[6]. Five enzyme markers diagnostic for *B. bombina* and *B. variegata* (*Ldh*-1, *Mdh*-1, *Ck*, *Ak* and *Gpi*) were analyzed by starch gel electrophoresis[17].

Using 17 restriction endonucleases, we compared mtDNA from one *B. bombina* and one *B. variegata*, collected in Żory and Stawek Zawadowski, respectively, far from the hybrid zone. Many more individuals from each locality were also analyzed, although not as extensively; no interindividual variation in mtDNA restriction sites was found at either locality. All but one restriction enzyme produced different fragment patterns in *B. bombina* and *B. variegata;* the species share 21% of 124 fragments[18], corresponding to a sequence divergence[19] of $9.4 \pm 1.1\%$. *B. bombina* and *B. variegata* are thus well differentiated in their mtDNAs, just as they are in their nuclear genes (Nei's $D = 0.49 \pm 0.13$)[20], morphology[21], anatomy[22,23], ecology[24] and breeding behavior[15,16].

Two unrelated restriction enzymes, Bcl I and Hind III, were used to identify the mtDNA type present in 141 individuals representing seven populations from a transect across the hybrid zone. Nuclear genotypes of the same individuals were also assessed (table). The distribution of bombina and variegata mtDNA types closely parallels that of nuclear genes (fig. 2). Three centralmost populations, Kopanka, Podbory Skawińskie and Skawina, had mtDNA of both species, although individuals always had one or the other mtDNA type only. A variant bombina mtDNA identified with Bcl I was found at Kopanka (2 individuals) and Podbory Skawińskie (3 individuals). This variant had a fragment 8800 bp long rather than the 5000 and 3800 bp fragments present in the typical bombina mtDNA. That the frequency of bombina mtDNA in samples from two consecutive years is similar, both at Kopanka and at Podbory Skawińskie, suggests that, in each case, these values are good estimates of the frequency in the population. Individuals from hybrid populations further to the south, Wola Radziszowska and Lanckorona, variegata-like in their nuclear genes, had only variegata mtDNA. Virtually all individuals from two hybrid populations to the north of the center, Tyniec and Rząska, had bombina mtDNA; only a single individual was found with variegata mtDNA. These two populations had a large preponderance of bombina alleles at nuclear genes. As expected, pure populations of *B. bombina* and *B. variegata* had exclusively their appropriate mtDNA types.

Analysis of nuclear genotypes revealed that all but one individual collected in the center of the zone (Kopanka, Podbory Skawińskie and Skawina) were hybrids. At Podbory Skawińskie, two F1-like individuals, heterozygous at all five loci, were found; one had bombina, the other variegata mtDNA. A single F1-like individual with bombina mtDNA was also found in Skawina.

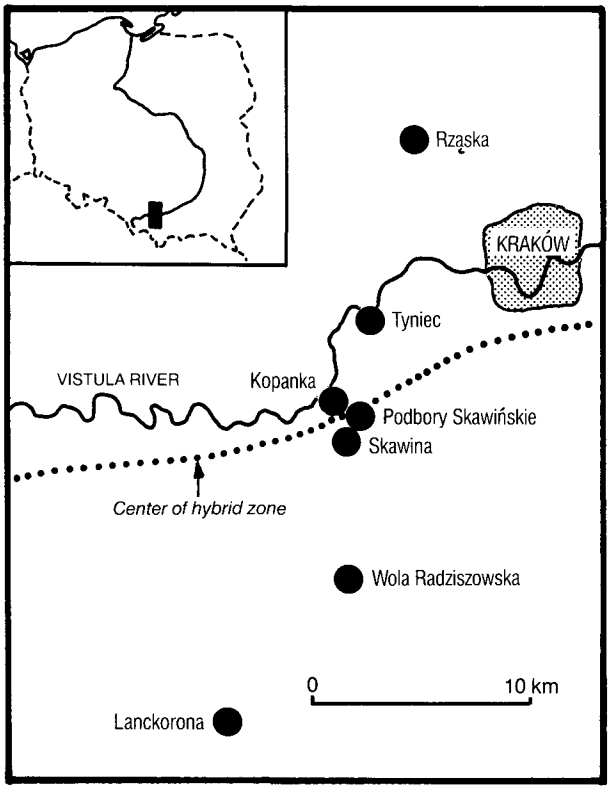

Figure 1. Distribution of populations sampled across a hybrid zone between *Bombina bombina* and *B. variegata* near Kraków, Poland.

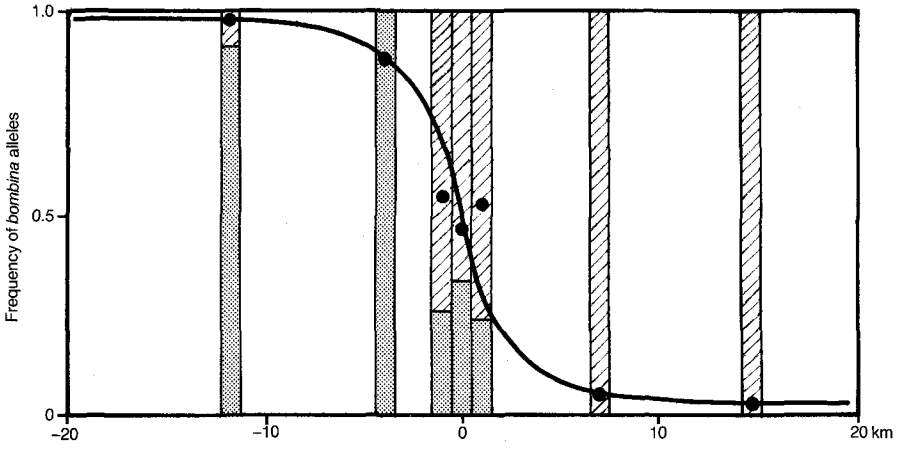

Figure 2. Patterns of change in mtDNA and nuclear alleles across a hybrid zone between *B. bomina* and *B. variegata*. The curved line approximates the frequency of bombina alleles across the zone; the closed circles indicate the bombina allele frequencies in the populations studied; the vertical bars indicate mtDNA frequencies; stippled = bombina mtDNA; cross-hatched = variegata mtDNA.

The only prospective non-hybrid, a frog homozygous for five *B. bombina* markers, was found in Kopanka; it had bombina mtDNA. Such individuals are expected to segregate in this population at a frequency of 0.002, which suggests that this individual had immigrated from an adjacent *B. bombina* population. An analysis of the frequency of bombina nuclear alleles within bombina and variegata mtDNA lineages, for all populations, revealed no significant association between nuclear and mitochondrial genomes; an initial association of bombina nuclear and mitochondrial alleles at Kopanka disappeared when the one apparently immigrant *B. bombina* individual was omitted from the calculation.

The frequency of bombina mtDNA in hybrid populations is opposite that expected on the basis of mating preferences in initial hybridizations; since mating barriers break down in populations composed almost exclusively of hybrids, this is not entirely surprising. In general, the frequency of bombina mtDNA in hybrid populations is lower than the frequency of bombina nuclear genes. This small difference in frequencies may reflect only slight asymmetries around the center. On the other hand, the mtDNA distribution may be a result of *B. bombina* range expansion causing a shift in position of the hybrid zone. The presence of insular populations of *B. variegata* surrounded by populations of *B. bombina* in the Hungarian Basin[25] and west of Kraków[26] speaks in favor of *B. bombina* range expansion. In a moving hybrid zone, in which males migrate more than females, as happens in *Bombina*[27], one would expect *B. bombina* mtDNA to lag behind its nuclear genes[28]. This phenomenon would also explain the presence of residual variegata mtDNA in the bombina-like population form Rząska.

Distribution of mitochondrial genotypes and frequency of bombina alleles in pure populations of *B. bombina* and *B. variegata* and in populations from a hybrid zone near Kraków

	Population	N	Frequency of bombina mtDNA	Nuclear alleles
B. bombina	Żory	9	1.000	1.000
Hybrid zone	Rząska	13	0.923	0.962
	Tyniec	18	1.000	0.900
	Kopanka, 1982	20	0.250	0.515
	1983	16	0.250	0.575
	Podbory Skawińskie, 1983	8	0.375	0.463
	1984	22	0.318	0.541
	Skawina	13	0.231	0.531
	Wola Radziszowska	15	0.000	0.075
	Lanckorona	16	0.000	0.029
B. variegata	Stawek Zawadowski	9	0.000	0.000
	Stróże	3	0.000	0.000

The concordance of nuclear and mitochondrial genomes in the hybrid zone described here suggests that the fate of mtDNA in hybrid populations depends on factors in addition to the mating preference of the parental species; these may include sex ratio, species ratio, reproductive mode and differential dispersal rates of males and females.

Acknowledgments. Jan N. Rafiński, Magdalena Kutschera and Ewa Tylek collected the animals. Supported by grants from the National Science Foundation (DEB 81-11397) and the Smithsonian Institution Foreign Currency Program to T.U., from the Whitehall Foundation to C.S. and T.U., and from the Polish Academy of Sciences (MR-II/6) to J.M.S.

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0014-4754/85/111469-02$1.50 + 0.20/0