Mitochondrial DNA Reveals Formation of Nonhybrid Frogs by Natural Matings between Hemiclonal Hybrids[1]

*Hansjürg Hotz,**,† Peter Beerli,† and Christina Spolsky**

*Department of Ecology, Ethology and Evolution, University of Illinois; and †Zoologisches Museum, Universität Zürich

The European water frog *Rana esculenta* (RL), a natural hybrid between *R. ridibunda* (RR) and *R. lessonae* (LL), reproduces by hybridogenesis: haploid gametes usually contain an intact chromosome set of *R. ridibunda* (R); the *lessonae* nuclear genome (L) is lost from the germ line. Hybridity is restored in the next generation, via fertilization by syntopic *R. lessonae*. Matings between two hybrids (RL × RL) usually give inviable *R. ridibunda* (RR) progeny. The adult *R. ridibunda* subpopulation of Trubeschloo, a gravel pit in northern Switzerland, consists only of females. Fragment patterns for mitochondrial DNA (mtDNA) of these *R. ridibunda* were identical with those of syntopic *R. esculenta* and of local populations of *R. lessonae;* they differed from the patterns in eastern European populations of *R. lessonae* and of *R. ridibunda* mtDNAs (3.7% and 9.3% estimated sequence divergence, respectively). In contrast, mtDNAs of two *R. ridibunda* from an introduced Swiss population with both sexes, although different (2.7% divergence) from each other, were typical *R. ridibunda* rather than *R. lessonae* mtDNAs. These data, together with unisexuality, demonstrate conclusively that the all-female *R. ridibunda* population at Trubeschloo originated from matings between two *R. esculenta*. The formation of independently reproducing *R. ridibunda* populations via such hybrid × hybrid matings is precluded because progeny of these matings are unisexual. Recombination in the regenerated fertile *R. ridibunda* females, followed by matings with *R. lessonae*, nevertheless provides a mechanism for meiotic reshuffling of genetic material in *ridibunda* haplotypes that is not typically available in hemiclonal lineages.

Introduction

European water frogs (*Rana esculenta* complex) are of general evolutionary interest because natural hybrid lineages reproduce by a hybridogenetic (Schultz 1969) gametogenesis without meiotic recombination [Graf and Polls Pelaz (1989) provided a recent review]. *Rana esculenta* (genomic composition RL) are hybrids between *R. ridibunda* (RR) and *R. lessonae* (LL); they typically make haploid gametes that contain only an intact *R. ridibunda* chromosome set (R), the *lessonae* genome (L) being lost in the germ line. Somatic hybridity is restored in the next generation because these gametes (R) are fertilized by gametes (L) of the syntopic sexual host species, *R. lessonae* (fig. 1). In such populations (the L-E system; Uzzell and Berger 1975), hybrid × hybrid matings (RL × RL) usually lead to inviable *R. ridibunda* (RR) progeny.

Hybridogenetic water frog lineages are unique among natural clonally reproducing vertebrate hybrids, in that most such frog lineages contain both sexes. This is a coincidental result of the sex-determining mechanism (XX-XY, male heterogametic; Berger et al. 1988) and of the directionality of original hybridizations: new *R. esculenta*

1. Key words: mtDNA, hemiclonal reproduction, hybridogenesis, hybrids, *Rana esculenta, Rana ridibunda.*

Address for correspondence and reprints: Hansjürg Hotz, Zoologisches Museum, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

Mol. Biol. Evol. 9(4):610–620. 1992.

0737-4038/92/0904-0004$02.00

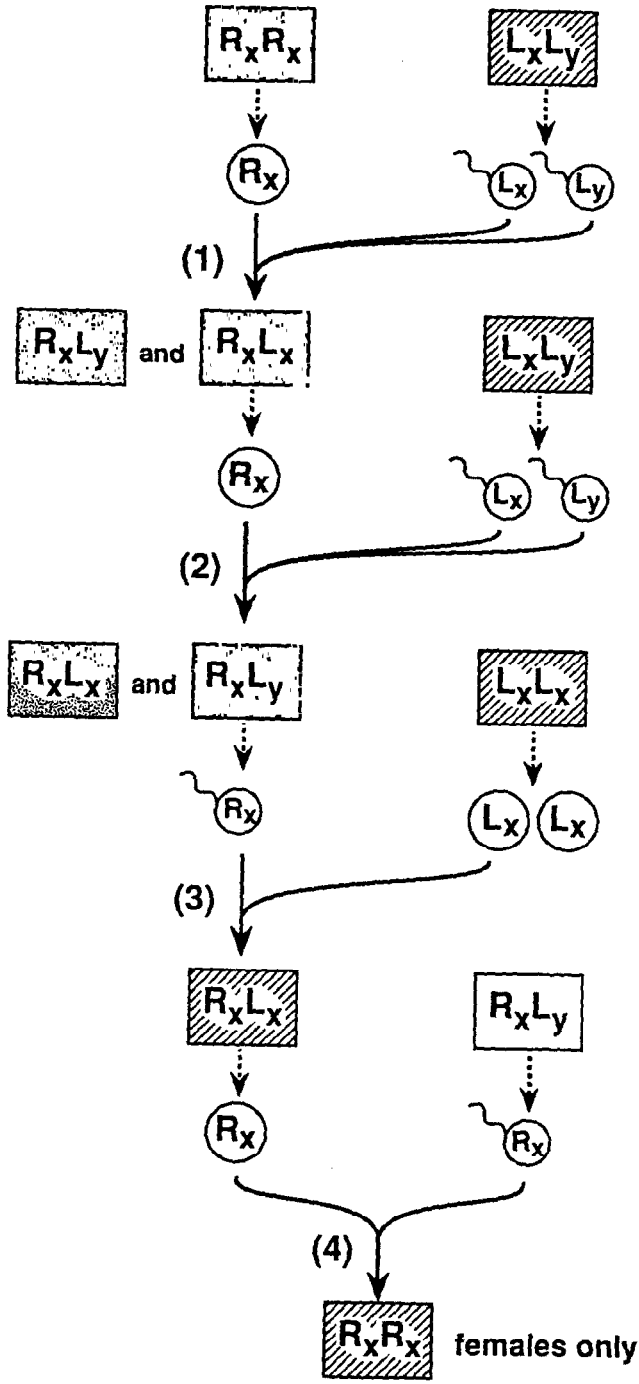

FIG. 1.—Overview of *Rana esculenta* L-E hybridogenetic system. Mating 1 is an interspecific hybridization establishing a hybridogenetic *R. esculenta* lineage; mating 2 is the usual way of maintaining an *R. esculenta* lineage; mating 3 (uncommon) irreversibly introduces *lessonae* mtDNA into an *R. esculenta* lineage; and mating 4 regenerates female *R. ridibunda*. [RR] = *R. ridibunda*; [LL] = *R. lessonae*; [RL] = *R. esculenta*; x and y = sex chromosomes associated with haploid genomes; ○ = ovum; ○ = sperm; ▩ = *ridibunda* mtDNA; and ▨ = *lessonae* mtDNA.

lineages are founded by matings between females of the large species *R. ridibunda* and males of the small species *R. lessonae,* the reciprocal being virtually precluded, in nature, because of behavioral reasons (see Tunner 1974; Berger et al. 1988). The clonally transmitted *ridibunda* genomes of natural *R. esculenta* lineages thus contain no male determinants, and sex of hybrids is determined by the *lessonae* genome.

A second consequence of the directionality of original hybridizations is that the maternally transmitted mitochondrial DNA (mtDNA) of newly formed hybrid lineages derives from *R. ridibunda* (fig. 1, mating 1). Once established, *R. esculenta* lineages are maintained predominantly, but not exclusively, by matings (fig. 1, mating 2) between female *R. esculenta* and male *R. lessonae* (Blankenhorn 1974, 1977; L. Berger, personal communication). In spite of this usual reproductive pattern, *R. esculenta* from L-E systems often have *lessonae* rather than the expected *ridibunda* mtDNAs (Spolsky and Uzzell 1986; also see Monnerot et al. 1984, 1986). Likewise, the hybridogenetic hybrids throughout Italy (see Uzzell and Hotz 1979) have *lessonae* rather than *ridibunda* mtDNA (H. Hotz, C. Spolsky, and T. Uzzell, unpublished results). Spolsky and Uzzell (1986) interpreted this reversal of mtDNA genotype as resulting from occasional successful matings between female *R. lessonae* and male *R. esculenta* (fig. 1, mating 3); for an *R. esculenta* lineage, the introduction of *lessonae* mtDNA by such a mating is irreversible. Moreover, a significant proportion of *R. ridibunda* in eastern Europe carry *lessonae* mtDNA (Spolsky and Uzzell 1984); this introgression of *R. lessonae* mtDNA into *R. ridibunda* was postulated to result from matings between *R. esculenta* females carrying *lessonae* mtDNA and either *R. ridibunda* or *R. esculenta* males (Spolsky and Uzzell 1986).

Although no native *R. ridibunda* occur in Switzerland, *R. ridibunda* from various geographic areas have been repeatedly introduced into several regions of Switzerland (see Grossenbacher 1988). Trubeschloo, a gravel pit in northern Switzerland, contains an adult water frog population composed of both sexes of *R. esculenta,* only females of *R. ridibunda,* and a few *R. lessonae.* Unisexuality, together with electrophoretic and skeletochronological results (Beerli 1986; P. Beerli and H. Hotz, unpublished results), suggests that the all-female *R. ridibunda* subpopulation was not introduced from elsewhere but originated in situ from natural matings among *R. esculenta.*

The matrilineality of metazoan mtDNA provides a tool (Wilson et al. 1985; Avise 1986; Avise et al. 1987; Moritz et al. 1987) for further discriminating between two alternative explanations for the occurrence of adult *R. ridibunda* at Trubeschloo: (1) a trivial one, i.e., introduction by humans, and (2) an evolutionarily significant one, i.e., successful matings between pairs of hybridogenetic *R. esculenta.* We tested these hypotheses by comparing mtDNAs of *R. ridibunda* and *R. esculenta* from Trubeschloo with those of *R. ridibunda* from a Swiss population known to be introduced; we also compared mtDNAs from these two populations to mtDNAs of *R. lessonae* from a nearby Swiss population, and to *R. lessonae* and *R. ridibunda* mtDNAs from native populations in central Poland. Our mtDNA results provide direct evidence for regeneration of adult *R. ridibunda* from natural matings between hemiclonally reproducing hybrids.

Material and Methods

Adult frogs were collected from four localities in Switzerland and Poland. At the gravel pit Trubeschloo (Beerli 1986) near Frauenfeld, 40 km northeast of Zürich, we collected three *Rana esculenta* in 1986 and four *R. esculenta* and three *R. ridibunda* in 1987. From an introduced population in a gravel pit near Embrach, 15 km north

Table 1
Restriction-Fragment Patterns of *Rana* mtDNAs

	Approximate Fragment Size (bp)					
	R. ridibunda			*R. ridibunda/ R. esculenta*	*R. lessonae*	
Enzyme	Poznań (N = 3)	Embrach 1 (N = 1)	Embrach 2 (N =1)	Trubeschloo (N = 3/7)	Frauenfeld (N = 1)	Poznań (N = 3)
*Ava*I	7,000[a]	7,000[a]	7,000[a]	8,900[a]	8,900[a]	7,000[a]
	3,800	3,800	3,800	6,900	6,900	5,800
	3,200	3,400	3,400	3,100	3,100	5,100
	2,000	3,200	3,200			1,300
	1,900	2,000	2,000			
	1,500					
*Bam*HI	19,500	19,500	No sites	12,500[a]	12,500[a]	11,000
				6,300	6,300	8,000[a]
				550	550	470
*Ban*II	3,800[a]	3,800[a]	3,800[a]	5,000[a]	5,000[a]	7,500[a]
	3,200	3,200	3,200	2,350	2,350	2,150
	2,150	2,150	2,100	2,200	2,200	2,100
	2,100	2,100	1,650	2,100	2,100	1,550
	1,650	1,650	1,400	1,750	1,750	1,400
	1,400	1,400	1,350	1,180	1,180	970
	1,350	1,350	1,180	1,150	1,150	930
	620	620	930	1,000	1,000	820
	550	550	900	760	760	760
	450	450	620	550	550	550
	410	410	550	410	410	410
	300	300	480	360	360	
	270	270	450			
			410			
			270			
*Bcl*I	11,500[a]	17,000[a]	10,000[a]	17,000[a]	17,000[a]	8,400[a]
	5,200	2,300	4,400	2,300	2,300	7,800
	2,300		2,500			2,300
	270		1,250			850
			1,050			
*Bgl*II	10,000[a]	10,000[a]	10,000[a]	5,300[a]	5,300[a]	5,800[a]
	3,550	5,200	3,200	3,700	3,700	3,700
	2,200	2,200	2,350	3,000	3,000	3,600
	1,800	1,800	1,800	1,800	1,800	3,000
	1,750		1,750	1,700	1,700	1,800
			350	1,200	1,200	1,650
				1,150	1,150	
				700	700	
				550	550	
*Cla*I	19,500	19,500	12,500	12,000	12,000	19,500
			7,000	7,200	7,200	
*Eco*RV	11,000	11,000	19,500	No sites	No sites[b]	16,000[a]
	8,600[a]	8,600[a]				3,600
*Hae*II	9,400	9,400	5,000[a]	8,900	8,900	8,900
	5,000[a]	5,000[a]	5,000	5,000[a]	5,000[a]	5,000[a]
	3,900	3,900	4,400	4,400	4,400	4,400
	1,120	1,120	3,900	640	640	640
			1,120	500	500	500

Table 1 (*Continued*)

Enzyme	Approximate Fragment Size (bp)					
	R. ridibunda			*R. ridibunda/ R. esculenta*	*R. lessonae*	
	Poznań (N = 3)	Embrach 1 (N = 1)	Embrach 2 (N =1)	Trubeschloo (N = 3/7)	Frauenfeld (N = 1)	Poznań (N = 3)
*Hin*dIII	5,600	5,600	5,600	5,600	5,600	5,600
	4,600	4,600	4,300	3,900	3,900	3,900
	4,200[a]	4,200[a]	4,200[a]	3,600[a]	3,600[a]	3,600[a]
	2,250	2,250	2,250	2,250	2,250	2,250
	1,330	1,330	1,330	1,600	1,600	1,600
	1,220	1,220	1,220	1,220	1,220	1,220
	580	580	580	580	580	580
			290	290	290	290
				220	220	220
*Kpn*I	13,000	13,000	13,000	8,600	8,600	8,600
	5,600[a]	5,600[a]	5,600[a]	5,200[a]	5,200[a]	5,200[a]
	680	680	680	3,400	3,400	3,400
				1,350	1,350	1,550
				680	680	680
*Nsi*I	19,500	No sites	No sites	19,500	19,500	11,000[a]
						5,600
						2,700
*Pst*I	7,100[a]	12,500	12,500	12,500	12,500	12,500
	6,200	7,100[a]	7,100[a]	4,200	4,200	7,100[a]
	6,100			2,400[a]	2,400[a]	
*Pvu*II	9,000	9,000	14,800[a]	15,000[a]	15,000[a]	15,000[a]
	5,800[a]	5,800[a]	3,400	4,400	4,400	2,400
	3,400	3,400	900			1,800
	900	900	400			320
	400	400				
*Spe*I	12,000[a]	12,000[a]	12,000[a]	8,700[a]	8,700[a]	9,800[a]
	2,400	2,400	2,250	4,400	4,400	3,500
	2,250	2,250	1,650	3,500	3,500	2,300
	1,650	1,650	1,400	1,500	1,500	2,250
	1,350	1,350	1,350	1,500	1,500	1,650
			1,000			
*Sph*I	No sites	No sites	19,500	19,500	19,500	19,500
*Ssp*I	3,700	3,700	6,800[a]	5,800[a]	5,800[a]	5,800[a]
	3,300[a]	3,300[a]	3,700	2,900	2,900	2,600
	1,900	1,900	2,600	2,600	2,600	2,200
	1,850	1,850	1,900	1,400	1,400	1,400[d]
	1,600	1,600	950	1,200[c]	1,200	1,250
	1,450	1,450	950	920	920	1,000
	1,400	1,400	830	860	860	950
	950	950	820	820	820	860
	950	950	540	650	650	820
	820	820		510	510	650
	540	630		430	430	620
				400	400	430
				325	325	400
*Sst*II	17,600[a]	17,600[a]	17,600[a]	17,600[a]	17,600[a]	17,600[a]
	1,700	1,700	1,700	1,700	1,700	1,700

Table 1 (*Continued*)

Enzyme	Approximate Fragment Size (bp)					
	R. ridibunda			*R. ridibunda/ R. esculenta*	*R. lessonae*	
	Poznań ($N = 3$)	Embrach 1 ($N = 1$)	Embrach 2 ($N = 1$)	Trubeschloo ($N = 3/7$)	Frauenfeld ($N = 1$)	Poznań ($N = 3$)
*Stu*I	8,800[a]	8,800[a]	8,800[a]	8,500[a]	8,500[a]	8,500[a]
	5,400	6,000	5,400	4,500	4,500	4,500
	3,800	3,800	3,200	3,200	3,200	3,200
	980	980	980	850	850	850
	520		620	700	700	700
			590	620	620	620
				520	520	520
				440	440	440
*Sty*I	3,400[a]	3,400[a]	3,400[a]	3,600	3,600	4,300[a]
	2,500	2,500	2,500	3,400[a]	3,400[a]	3,600
	2,000	2,000	2,300	2,150	2,150	2,900
	1,900	1,650	1,850	1,900	1,900	1,900
	1,650	1,350	1,800	1,450	1,450	1,250
	1,350	1,300	1,650	1,250	1,250	1,050
	1,300	1,120	1,120	860	860	890
	1,120	1,100	840	840	840	840
	1,100	840	750	640	640	780
	630	750	540	610	610	630
	540	630	520	520	520	520
	530	540	500	500	500	310
	520	530	470	420	420	
	470	520	370	360	360	
	270	490	200	300	300	
	190	470	190	190	190	
		270		170	170	
		190				

[a] Length-variable fragment (approximate average size is shown). Total genome size may vary between taxa but could not be accurately estimated.

[b] No site on the mtDNA type D of the Swiss water frogs previously reported to have one site (Spolsky and Uzzell 1986).

[c] Three individuals (two *R. ridibunda* and one *R. esculenta*) showed a 1,350-bp *Ssp*I fragment instead.

[d] One individual showed a 1,700-bp *Ssp*I fragment instead.

of Zürich, we collected two *R. ridibunda.* For comparison we used both *R. lessonae* from Frauenfelder Allmend near Frauenfeld, Switzerland (~10 km from Trubeschloo) and *R. ridibunda* and *R. lessonae* mtDNAs from the environs of Poznań, Poland.

Isolation and purification of mtDNA from each individual followed standard methods (Spolsky and Uzzell 1984). We used 19 hexanucleotide-recognizing restriction endonucleases (table 1) to digest each mtDNA. Restriction-enzyme-fragment patterns were determined from autoradiographs of ^{32}P-end-labeled digests after electrophoresis through horizontal 0.5%–1.6% agarose gels. To ascertain fragment homology, some restriction sites, including those of all enzymes that only once cleaved mtDNA of more than one sample (*Bam*HI, *Cla*I, *Nsi*I, and *Sph*I), were mapped using double digests; for these we used the additional enzyme *Apa*LI. Fragment sizes were estimated using DNA fragments of known lengths on each gel (*Hin*dIII-restricted λ DNA and a 1-kb ladder from BRL).

Sequence divergence between pairs of mtDNAs was estimated as the percent of sites that differ from the proportion of shared restriction fragments (Nei and Li 1979), by using Upholt's (1977) formula. From the sequence divergences, we generated a tree of relationships by using the FITCH program in Felsenstein's (1985) program package PHYLIP.

Results

The seven *Rana esculenta* and three *R. ridibunda* examined from Trubeschloo had identical restriction fragment patterns for 18 of the 19 endonucleases used (tables 1 and 2). Two mtDNA haplotypes are distinguished by *Ssp*I; they have been observed both in *R. ridibunda* and in *R. esculenta* from Trubeschloo (table 1). The two haplotypes are very similar: they share a total of 101 restriction sites and differ by <0.1% estimated sequence divergence. The patterns of one of the two haplotypes are identical to those of mtDNAs of *R. lessonae* from the nearby Swiss locality Frauenfeld (table 1). The mtDNA patterns of the Trubeschloo frogs differ from those of Polish *R. lessonae* populations, however, by an estimated sequence divergence of 3.7% (table 2). Distances to nonintrogressed (see Spolsky and Uzzell 1984) mtDNAs of native central Polish *R. ridibunda* are even greater, amounting to a sequence divergence of 9.3% (table 2). mtDNAs of *R. ridibunda* and *R. esculenta* from Trubeschloo clearly cluster with mtDNAs of *R. lessonae* and not with those of *R. ridibunda* (fig. 2).

For many restriction enzymes, one mtDNA fragment contained a length-variable region. In otherwise identical fragment patterns, this fragment showed both intra- and interindividual size variation. Such variation is visible in profiles of most enzymes that generate more than one fragment as a diffuse or multibanded region (fig. 3). This variable region is present in all mtDNAs examined; other than the two *Ssp*I haplotypes, it is the only exception to the fragment-pattern identity of all Trubeschloo frogs. Because such length variations, which are not genealogically stable (see Moritz et al. 1987; Rand and Harrison 1989), are not caused by gain or loss of restriction sites, and because the fragment containing the length-variable region is defined by two homologous restriction sites, such fragments are considered homologous.

In contrast to the largely homogeneous mtDNAs in *R. ridibunda* from Trubeschloo, mtDNAs of the two *R. ridibunda* from Embrach, although both most similar

Table 2
mtDNA Sequence Divergence Values

	mtDNA Sequence Divergence between Taxa (% of nucleotides different)					
Taxon (locality)	1	2	3	4	5	6
1: *Rana esculenta* (Trubeschloo)						
R. ridibunda (Trubeschloo)		00.0	03.7	09.3	09.2	08.0
2: *R. lessonae* (Switzerland)[a]			03.7	09.3	09.2	08.0
3: *R. lessonae* (Poland)[b]				07.7	07.5	07.3
4: *R. ridibunda* (Poland)[c]					00.8	02.9
5: *R. ridibunda* (Embrach 1)						02.7
6: *R. ridibunda* (Embrach 2)						

[a] Very similar to mtDNA type D (Spolsky and Uzzell 1986).
[b] mtDNA type C (Spolsky and Uzzell 1984).
[c] mtDNA type A (Spolsky and Uzzell 1984).

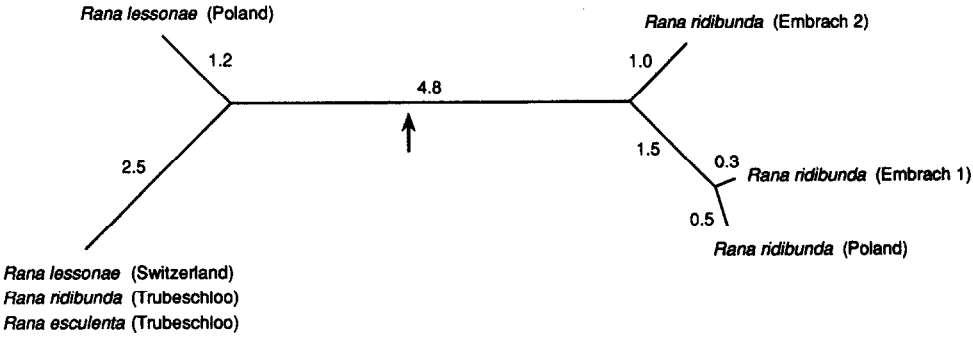

FIG. 2.—FITCH tree (Felsenstein 1985) of phylogenetic relationships, based on mtDNA sequence divergences among six populations of *Rana esculenta* complex. The tree is unrooted; a tentative position for a root at the midpoint of the longest distance is indicated by the arrow. Numbers along branches indicate relative branch lengths and are proportional to sequence divergences in table 1.

to other *ridibunda,* rather than *lessonae,* mtDNAs (fig. 2), differed from each other by ~2.7% (table 2). mtDNA of one individual from Embrach was similar to that of *R. ridibunda* from Poland (0.8% sequence divergence), but mtDNA of the other differed from mtDNA of Polish *R. ridibunda* by 2.9% (table 2 and fig. 2).

Discussion

No native *Rana ridibunda* populations are known in Switzerland, the natural western edge of the species' range passing through regions well to the east and north of this country (see Günther 1990). *Rana ridibunda* has, however, been repeatedly introduced by humans into northern and western Switzerland, from eastern and southeastern Europe and Anatolia (Grossenbacher 1988). The genetic pattern of such introduced *R. ridibunda* populations, containing both sexes, is exemplified by the two frogs from Embrach: both have mtDNAs similar to *ridibunda,* rather than *lessonae,* mtDNAs (table 2 and fig. 2). Moreover, the 2.7% sequence divergence between their mtDNAs is much larger than the amount of intrapopulational mtDNA divergence usually observed (in the absence of interspecies transfers; Spolsky and Uzzell 1984) in this group of frogs (H. Hotz, C. Spolsky, and T. Uzzell, unpublished results; also see Spolsky and Uzzell 1984, 1986; Monnerot et al. 1986). This large difference is concordant with protein electrophoretic data: these two *R. ridibunda* individuals from Embrach are homozygous for different alleles at two of seven loci examined (P. Beerli and H. Hotz, unpublished results relating to LDH-B *a* and *c* and to MPI *a* and *c;* see Hotz and Uzzell 1982; Hotz 1983); this is compatible with their not originating from a single deme in Hardy-Weinberg equilibrium. Thus, protein data are consistent with the mtDNA data that show separate origins and independent introductions of these two frogs.

Data on the all-female *R. ridibunda* population from Trubeschloo are very different. These *R. ridibunda* appear to have the same *lessonae*-like mtDNA as do *R. esculenta* from Trubeschloo. Because some *R. ridibunda* in Poland carry an introgressed *lessonae*-like mtDNA, which was also present in an *R. ridibunda* from an introduced population in western Switzerland (Spolsky and Uzzell 1984; type B mtDNA), it seemed possible that the identity of mtDNA in *R. ridibunda* and *R. esculenta* at Trubeschloo resulted from exogeneous introduction of *R. ridibunda* carrying such *lessonae* mtDNA into this area of Switzerland, followed by the formation of new *R. esculenta* lineages by matings of such *R. ridibunda* females with *R. lessonae* males. It

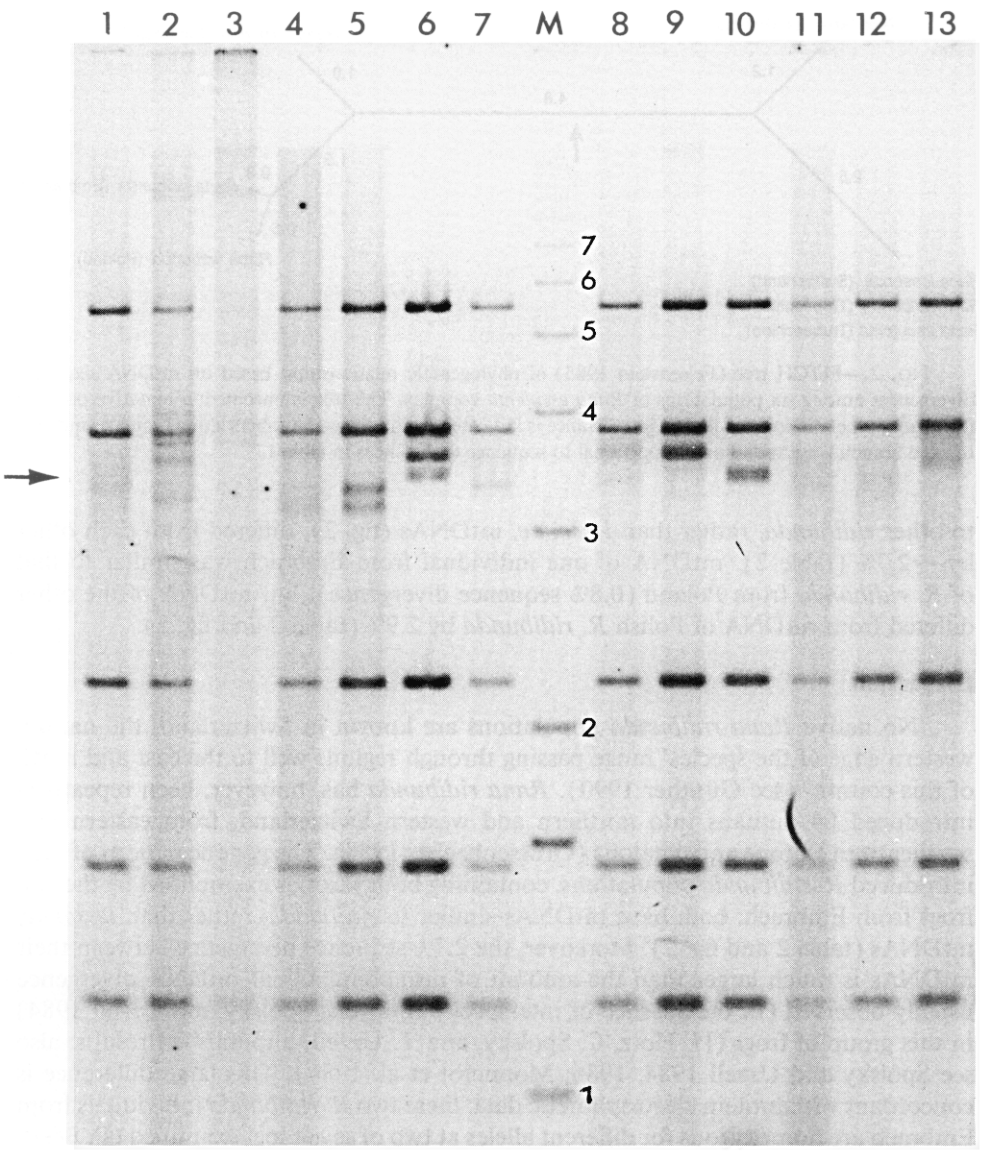

FIG. 3.—Autoradiogram of *Hin*dIII restriction-fragment patterns for mtDNAs of water frogs from northern Switzerland. Fragments were separated on a 0.7% agarose gel. Fragment lengths of the size marker (M; 1-kb ladder) are given in kilobases. The arrow indicates the region of the length-variable fragment. Lane 1, *Rana lessonae* from Frauenfeld. Lanes 2–4, *R. ridibunda* from Trubeschloo. Lanes 5–11, *R. esculenta* from Trubeschloo. Lane 12, *R. lessonae* from Frauenfeld. Lane 13, *R. lessonae* from Poznań. In this gel, much of material in lane 3 remained at the origin; the same six bands as in all other lanes were apparent, however, on the autoradiogram.

was therefore important to distinguish between mtDNAs of northern-Swiss and more-eastern populations of *R. lessonae*. mtDNA of the Trubeschloo frogs appears the same as mtDNA of northern Swiss *R. lessonae* from Frauenfeld but differs considerably (3.7%; table 2 and fig. 2) from *R. lessonae* mtDNAs from Poznań in central Poland. It thus is also distinct from the introgressed *R. ridibunda* mtDNA type B, which differs from Polish *R. lessonae* mtDNA (type C) by only 0.3% (Spolsky and Uzzell 1984).

These mtDNA data, in conjunction with unisexuality, confirm the origin of the Trubeschloo *R. ridibunda* population from *R. esculenta* × *R. esculenta* matings (fig. 1, mating 4). The only alternative explanation of the mtDNA results—i.e., reconstitution of *R. ridibunda* from matings between an *R. esculenta* female carrying *lessonae* mtDNA and an introduced *R. ridibunda* male—is incompatible with the observation that all 56 *R. ridibunda* for which sex was determined were female (P. Beerli and H. Hotz, unpublished data). All-female progeny are expected from hybrid × hybrid matings, whereas a 1:1 sex ratio is expected in *R. ridibunda* progeny from an *R. esculenta* female × *R. ridibunda* male mating (Berger et al. 1988). The conclusion agrees with independent protein electrophoretic data (P. Beerli and H. Hotz, unpublished results): the *R. ridibunda* at Trubeschloo showed only electrophoretic alleles occurring in *ridibunda* genomes of the Trubeschloo *R. esculenta* hemiclones and had significant excess heterozygosity, indicating that most successful hybrid × hybrid matings were interhemiclonal. That the two Trubeschloo mtDNA haplotypes distinguished by *Ssp*I both occur in *R. ridibunda* as well as in *R. esculenta* shows that *R. ridibunda* has been regenerated by more than one *R. esculenta* × *R. esculenta* mating.

No independently reproducing *R. ridibunda* populations can be founded by such hybrid × hybrid matings, because these matings produce all-female progeny. The mature *R. ridibunda* females generated this way can, however, lead to another potentially important evolutionary consequence. Their two *ridibunda* genomes are expected to recombine in a normal Mendelian meiosis. In contrast to gametes of a hybridogenetic *R. esculenta,* ova of an *R. ridibunda* reconstituted from an interhemiclonal hybrid × hybrid mating may contain a variety of different genotypes; the amount of generated diversity depends on the genetic difference between the *ridibunda* haplotypes of the source hemiclones. When such *R. ridibunda* mate with *R. lessonae,* new *R. esculenta* hemiclones can be formed, and *ridibunda* haplotypes freed from deleterious recessive alleles can be generated.

Acknowledgments

We thank Gaston Guex (Zurich) for help in capturing frogs and purifying mtDNA. We thank Thomas Uzzell, Christopher Phillips, and Gregory Whitt for critical comments and suggestions on earlier versions of the manuscript, and we thank three anonymous reviewers for helpful comments. This research was supported by U.S. National Science Foundation grants BSR 86-14881 (to H.H.H. and T.U.) and BSR 88-18630 (to C.S.).

LITERATURE CITED

AVISE, J. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. Lond. [B] **312**:325–342.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, and N. C. SAUNDERS. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. **18**:489–522.

BEERLI, P. 1986. Seefrösche in einer Tümpelfrosch-Wasserfrosch-Population in einem anthropogen beeinflussten Habitat. Diplomarbeit, Universität Zürich, Zurich.

BERGER, L., T. UZZELL, and H. HOTZ. 1988. Sex determination and sex ratios in western Palearctic water frogs: XX and XY female hybrids in the Pannonian Basin? Proc. Acad. Natl. Sci. Philadelphia **140**:220–239.

BLANKENHORN, H. 1974. Soziale Organisation einer Mischpopulation von *Rana lessonae* Camerano und *Rana esculenta* Linnaeus. Ph.D. dissertation, Universität Zürich, Zurich.

———. 1977. Reproduction and mating behavior in *Rana lessonae-Rana esculenta* mixed

populations. Pp. 389–410 *in* D. H. TAYLOR and S. I. GUTTMAN, eds. The reproductive biology of amphibians. Plenum, New York and London.

FELSENSTEIN, J. 1985. Confidence limits in phylogenies: an approach using the bootstrap. Evolution **39**:783–791.

GRAF, J.-D., and M. POLLS PELAZ. 1989. Evolutionary genetics of the *Rana esculenta* complex. Pp. 289–302 *in* R. M. DAWLEY and J. P. BOGART, eds. Evolution and ecology of unisexual vertebrates. N.Y. State Museum Bull. 466. The University of the State of New York, Albany.

GROSSENBACHER, K. 1988. Verbreitungsatlas der Amphibien der Schweiz. Doc. Faunistica Helv. **7**:1–207.

GÜNTHER, R. 1990. Die Wasserfrösche Europas (Anura-Froschlurche). Neue Brehm-Bücherei 600, Wittenberg.

HOTZ, H. 1983. Genic diversity among water frog genomes inherited with and without recombination. Ph.D. dissertation, Universität Zürich, Zurich.

HOTZ, H., and T. UZZELL. 1982. Biochemically detected sympatry of two water frog species: two different cases in the Adriatic Balkans (Amphibia, Ranidae). Proc. Acad. Natl. Sci. Philadelphia **134**:50–79.

MONNEROT, M., A. DUBOIS, and H. TUNNER. 1986. Mitochondrial DNA polymorphism among *Rana ridibunda, Rana lessonae* and *Rana* kl. *esculenta:* preliminary study. Alytes **4**:101–112.

MONNEROT, M., J.-C. MOUNOLOU, and M. SOLIGNAC. 1984. Intra-individual length heterogeneity of *Rana esculenta* mitochondrial DNA. Biol. Cell **52**:213–218.

MORITZ, C., T. E. DOWLING, and W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18**:269–292.

NEI, M., and W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76**:5269–5273.

RAND, D. M., and R. G. HARRISON. 1989. Molecular population biology of mtDNA size variation in crickets. Genetics **121**:551–569.

SCHULTZ, R. J. 1969. Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Natl. **103**:605–619.

SPOLSKY, C., and T. UZZELL. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proc. Natl. Acad. Sci. USA **81**:5802–5805.

———. 1986. Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. Mol. Biol. Evol. **3**:44–56.

TUNNER, H. G. 1974. Die klonale Struktur einer Wasserfroschpopulation. Z. Zool. Syst. Evol. Forsch. **12**:309–314.

UPHOLT, W. B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Res. **4**:1257–1265.

UZZELL, T., and L. BERGER. 1975. Electrophoretic phenotypes of *Rana ridibunda, Rana lessonae,* and their hybridogenetic associate, *Rana esculenta.* Proc. Acad. Natl. Sci. Philadelphia **127**: 13–24.

UZZELL, T., and H. HOTZ. 1979. Electrophoretic and morphological evidence for two forms of green frogs (*Rana esculenta* complex) in peninsular Italy (Amphibia, Salientia). Mitt. Zool. Museum Berlin **55**:13–27.

WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, and M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linnean Soc. **26**:375–400.

WESLEY M. BROWN, reviewing editor

Received March 13, 1991; revision received November 25, 1991

Accepted January 11, 1992