Ecology Letters, (2001) **4**: 628–636

REPORT

Non-hybrid offspring from matings between hemiclonal hybrid waterfrogs suggest occasional recombination between clonal genomes

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Abstract

The hemiclonal waterfrog *Rana esculenta*, a hybrid between *R. ridibunda* and *R. lessonae*, eliminates the *lessonae* genome from the germline and clonally transmits the *ridibunda* genome (hybridogenesis). Such genomes are prone to accumulate deleterious mutations, which may explain why offspring from matings between hybrids are typically inviable. Here I present field data from a population for which experimental crossings showed that some *R. esculenta* pairs produce viable *R. ridibunda* offspring. I demonstrate: (1) that *R. ridibunda* metamorphs are also produced and survive under natural conditions; (2) that their genotypes are consistent with combinations of clonal *ridibunda* genomes found in hybrids; and (3) that all *R. ridibunda* are female. These females possibly recombine the clonal genomes they inherited and, upon mating with syntopic *R. lessonae*, produce new hemiclones with novel combinations of alleles. Hence, occasional recombination between otherwise clonal *ridibunda* genomes seems plausible and may provide an escape from the evolutionary dead end they were proposed to be trapped in.

Keywords

Clonal reproduction, deleterious mutations, hybridogenesis, *Rana esculenta* complex, recombination.

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INTRODUCTION

The evolutionary longevity of clones has been the subject of much debate in the field of evolutionary biology (e.g. Lynch & Gabriel 1990; Maynard Smith 1992; Butlin *et al.* 1999; Welch & Meselson 2000). Although there are some important exceptions (reviewed by Judson & Normark 1996), the taxonomic distribution of asexuals suggests that they are generally short-lived on an evolutionary timescale (Maynard Smith 1978). This is surprising because, all else being equal, parthenogens have a twofold advantage over sexuals (Maynard Smith 1978). But all else is generally not equal. Some parthenogens suffer from reduced fertility, fecundity or viability compared to their sexual relatives (e.g. Corley & Moore 1999). Furthermore, their long-term evolutionary success may be impaired by an inability to cope with rapidly changing environments and a tendency to accumulate slightly deleterious mutations (Kondrashov 1988; Maynard Smith 1988). However, theoretical studies show that only occasional recombination is sufficient to largely avoid these disadvantages (Lynch & Gabriel 1983; Charlesworth *et al.* 1993; Green & Noakes 1995; Hurst & Peck 1996). It has therefore been suggested that many seemingly asexual organisms are not truly asexual, but exhibit some form of 'covert sex' that has so far been overlooked (Hurst *et al.* 1992; Little & Hebert 1996). Here I present data indicating that such occasional recombination may occur between otherwise clonally transmitted genomes in the hybridogenetic waterfrog *Rana esculenta* L.

Hybridogens are hemiclonal interspecific hybrids that eliminate the genome of one parental species from the germline prior to meiosis and clonally transmit the genome of the other parental species (Schultz 1969). They can persist in mixed populations by backcrossing with the parental species whose genome they exclude, regenerating the hybrid genotype in the next generation. Hybridogens can thus be regarded as sexual parasites. They possess one clonal genome that they inherit from their hybrid parent, and one sexual genome that they 'borrow' from their parental species for only one generation. This peculiar reproductive mode has so far been reported from stick insects of the genus *Bacillus* (Mantovani & Scali 1992), from teleost fish of the genera *Poeciliopsis* and *Tropidophoxinellus* (Schultz 1969; Carmona *et al.* 1997) and from waterfrogs of the genus *Rana* (reviewed by Graf & Polls Pelaz 1989).

Rana esculenta is a bisexual hybrid between *R. ridibunda* Pallas and *R. lessonae* Camerano (Berger 1967, 1968). It eliminates the *lessonae* genome from the germline and clonally transmits the *ridibunda* genome. Over large parts of central Europe, including Switzerland, *R. esculenta* occurs in mixed populations with its sexual host *R. lessonae*, forming the so-called L-E-system (Fig. 1A; Uzzell & Berger 1975). It is assumed that hybrids have immigrated from eastern Europe where *R. ridibunda* and *R. lessonae* are sympatric and recurrent hybridization is possible. In the L-E-system, matings between hybrids produce non-hybrid offspring possessing two clonal *ridibunda* genomes. However, such offspring typically die at an early larval stage (Fig. 1A), because they express recessive deleterious mutations (Berger & Uzzell 1977; Binkert *et al.* 1982; Semlitsch & Reyer 1992; Vorburger 2001). Such mutations may have pre-existed in the original sexual *R. ridibunda* population and become 'frozen' in clonal genomes through hybridization, and/or have accumulated after hemiclone formation due to the lack of recombination (Vorburger, in press). Similarly, clonal *monacha* genomes of the hybridogenetic fish *Poeciliopsis monacha-lucida* and *Poeciliopsis monacha-occidentalis* suffer from the accumulation of deleterious mutations through Muller's ratchet (Leslie & Vrijenhoek 1978, 1980).

Considering that a little sex may be as good as a lot in the struggle against spontaneous deleterious mutations and changing environments, cyclical parthenogenesis, as seen for example in aphids, cladocerans and rotifers, may represent an optimal compromise, combining the benefits of sexual and asexual reproduction, while minimizing the inevitable costs of both (Hastings 1992). After Hotz *et al.* (1992) had published their finding that non-hybrid *R. ridibunda* from natural matings between hemiclonal *R. esculenta* occur in a Swiss population, Schmidt (1993) suggested that the reproductive system of *R. esculenta* is comparable to cyclical parthenogenesis and may thus provide similar benefits. Due to the fact that clonal *ridibunda* genomes generally contain an X chromosome, successful matings between hybrids cannot found independently reproducing *R. ridibunda* populations as the resulting offspring are all females (Berger *et al.* 1988). Nevertheless, the formation of such *R. ridibunda* females may have important evolutionary consequences, because they are expected to exhibit normal Mendelian meiosis and thus to recombine the two clonal genomes they inherited (Fig. 1B). Renewed hybridizations with *R. lessonae* males would then found new *ridibunda* hemiclones possessing novel gene combinations, some of which may be purged from deleterious mutations present in the original hemiclones (Fig. 1B). Occasional recombination of this kind could provide an escape from the evolutionary dead end in

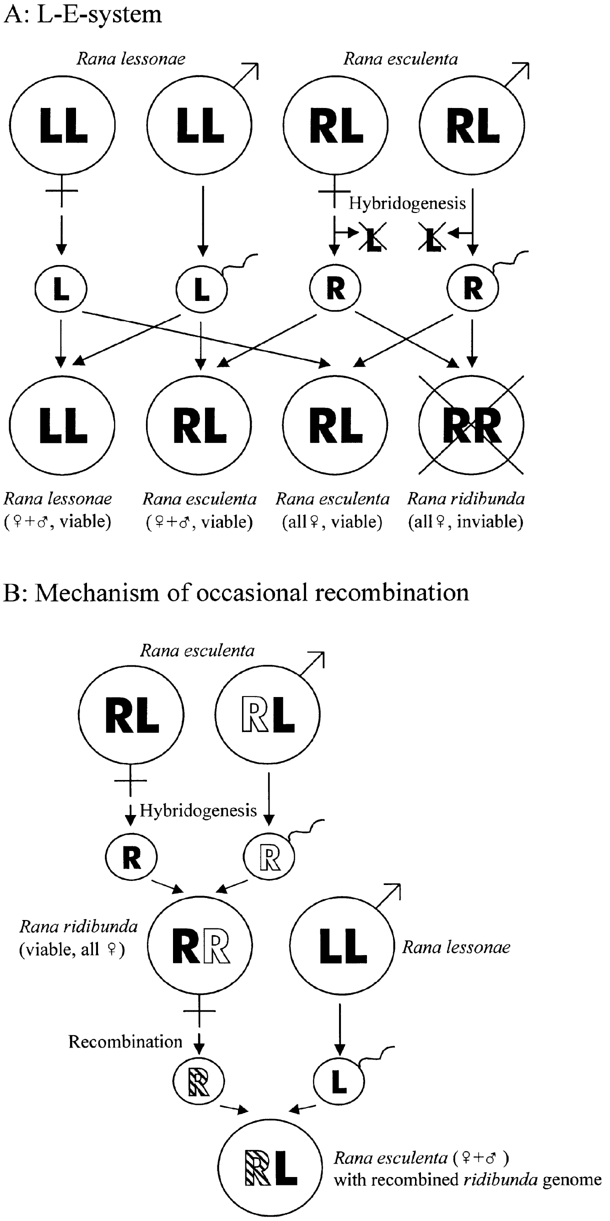

Figure 1 Mating combinations and resulting offspring in the L-E-system, i.e. mixed populations of *R. lessonae* and *R. esculenta* (A), and the proposed mechanism (Schmidt 1993) leading to occasional recombination between otherwise clonally transmitted *ridibunda* genomes (B). The hybrid *R. esculenta* excludes the *lessonae* (L) genome from the germline and produces gametes containing the unrecombined *ridibunda* (R) genome (hybridogenesis). Offspring from matings between hybrids are typically inviable (A), but can occasionally be viable if different, genetically compatible *ridibunda* genomes occur in a population (B). Resulting *R. ridibunda* offspring are all-female, because clonal *ridibunda* genomes contain an X-chromosome (Berger *et al.* 1988). Such females are expected to exhibit normal Mendelian meiosis and could thus produce new, recombinant hemiclones upon mating with sympatric *R. lessonae*.

which clonal *ridibunda* genomes were proposed to be trapped (Milinski 1994).

So far, the population described by Hotz *et al.* (1992) is the only well-documented case of the formation of female *R. ridibunda* from hybrid × hybrid matings in an L-E-system, but their population contained hybrids from different sources introduced by humans. However, episodic observations of *R. ridibunda* females or metamorphs have also been reported from undisturbed L-E-systems (Wijnands 1979; Grossenbacher 1988; Hotz *et al.* 2001). Based on extensive field sampling and genetic analyses in a natural, undisturbed population in Switzerland, I here provide conclusive evidence for the formation of viable, all-female *R. ridibunda* by matings between hemiclonal *R. esculenta*, suggesting that this phenomenon is more common than previously expected.

METHODS

Study site and field sampling

My study site is located in an area called Elliker Auen, near Ellikon in northern Kanton Zürich, Switzerland. The breeding habitat of the waterfrogs is adjacent to the rivers Rhine and Thur, and consists of several permanent ponds within ancient river oxbows. No formal estimate of the population size is available, but the population certainly contains many thousands of individuals. It is important to note that this population represents a native L-E-system, i.e. *R. ridibunda*, which does not occur naturally in Switzerland, has never been introduced into the population. Such introductions have occurred elsewhere in Switzerland (Grossenbacher 1988), but so far there are no reports of introduced *R. ridibunda* at or near Elliker Auen. The present study with more than 500 frogs collected over 3 years confirmed that this site is undisturbed, as no male *R. ridibunda* could be found.

During the breeding season in May 1998, frogs were captured on one occasion in order to obtain *R. esculenta* for a large crossing experiment including hybrids from three different populations (Vorburger, in press). Unexpectedly, this experiment revealed that matings between hybrids from Elliker Auen can produce offspring that are viable under benign conditions in artifical ponds: tadpoles from three out of five crosses successfully completed metamorphosis. To find out whether such *R. ridibunda* from hybrid × hybrid matings also occur under natural conditions, I increased the sampling effort over the following 2 years and collected adults and juveniles as well as newly metamorphosed froglets on several occasions in 1999 and 2000.

In 1999, peak metamorphosis was reached around 20 July, and the number of metamorphs produced in 1999 was enormous, because this year provided extremely favourable growth conditions for tadpoles. After heavy rainfalls in May, the area was partly flooded by the Rhine river, and all breeding ponds extended far beyond their usual borders into the surrounding fields for approximately 2 months. Thus, tadpoles experienced high food levels and low densities of both competitors and predators. In 2000, growth conditions for tadpoles were not as favourable, because breeding ponds were confined to their natural borders throughout most of the season. As a consequence, markedly fewer metamorphs emerged than in 1999, metamorphosis occurred about 3 weeks later with a peak around 10 August, and metamorphs were much smaller.

Allozyme analysis

I took toe clips from all metamorphs in 1999 and 2000, and from a subsample of the adult and juvenile frogs over all three years for cellulose acetate electrophoresis of enzymes following standard procedures (Hebert & Beaton 1993). Adults and juveniles were scored for variation at six loci previously known to be polymorphic in *R. ridibunda* (Hotz 1983): *sAAT*, *GPI*, *LDH-B*, *MPI*, *PGM-2* and *PGDH* (locus designations follow Hotz *et al.* 1997). It was possible to assign all alleles of individual *R. esculenta* to either their *ridibunda* or their *lessonae* genome. A hemiclone was then defined as a distinct haploid multilocus genotype of a *ridibunda* genome in *R. esculenta*. Metamorphs were first only analysed for variation at *LDH-B*, which exhibits fixed allelic differences between *R. ridibunda* and *R. lessonae*. Those metamorphs found to be *R. ridibunda* were subsequently gentoyped at all six loci in order to see whether their multilocus genotypes were consistent with combinations of hemiclones present in the *R. esculenta* population. The observed frequency distribution of multilocus genotypes in *R. ridibunda* was further tested against the expected distribution of all possible hemiclone combinations under the assumption of random mating among *R. esculenta* and equal survival probabilities of all offspring. Deviations from this null model would allow conclusions to be drawn as to which combinations of clonal genomes are viable or inviable, respectively.

Rearing of metamorphs

To monitor their survival and development after metamorphosis, 33 newly metamorphosed *R. ridibunda* and, for comparison, 11 metamorphs each of *R. lessonae* and *R. esculenta* were taken from the field to the University of Zürich in July 1999. Frogs were kept separated by species in indoor terraria containing 11 individuals each. Crickets and mealworms *ad libitum* were provided as food, the temperature ranged from 20 to 25 °C. Under these conditions, frogs developed quickly and could unambiguously be sexed already before their first hibernation from January to April 2000, when frogs were overwintered in a cool room at 5 °C. After hibernation, all surviving *R. lessonae* and *R. esculenta* were released at the capture site, and surviving *R. ridibunda* were kept at the University for further studies. However, I lost these animals during their second hibernation due to the outbreak of a bacterial infection (Red Leg disease). Thirteen *R. ridibunda* were subsequently dissected in order to examine the development of their ovaries.

RESULTS

Field captures

In 1998 and 1999, no adult or juvenile *R. ridibunda* were captured in the population, but out of 93 newly metamorphosed froglets I captured in 1999, 33 were *R. ridibunda*, 35 were *R. esculenta* and 25 were *R. lessonae* (Fig. 2). Due to the high number of metamorphs produced in 1999, a large fraction of the population consisted of one-year-old frogs in 2000. This resulted in a considerably smaller average size of both *R. esculenta* and *R. lessonae* than in the years before (Fig. 2). Variation in size among years was significant for both taxa (*R. esculenta*: $F_{2,252} = 46.42$, $P < 0.001$; *R. lessonae*: $F_{2,155} = 69.63$, $P < 0.001$). Scheffé's test indicated that *R. esculenta* and *R. lessonae* were significantly smaller in 2000 than in 1998 and 1999 (all $P < 0.001$), but that sizes did not differ significantly between 1998 and 1999 (*R. esculenta*: $P = 0.68$; *R. lessonae*: $P = 0.78$). In 2000, I also captured a total of 17 *R. ridibunda* (Fig. 2). Their snout-vent length (SVL) ranged from 32 to 57 mm, which is within the range for one-year-old *R. ridibunda* given in Günther (1990). Among 62 metamorphs captured in 2000, there were 51 *R. esculenta*, 11 *R. lessonae* and no *R. ridibunda* (Fig. 2).

Obviously, some *R. ridibunda* produced in 1999 survived the first winter, but their relative frequency within the 1999

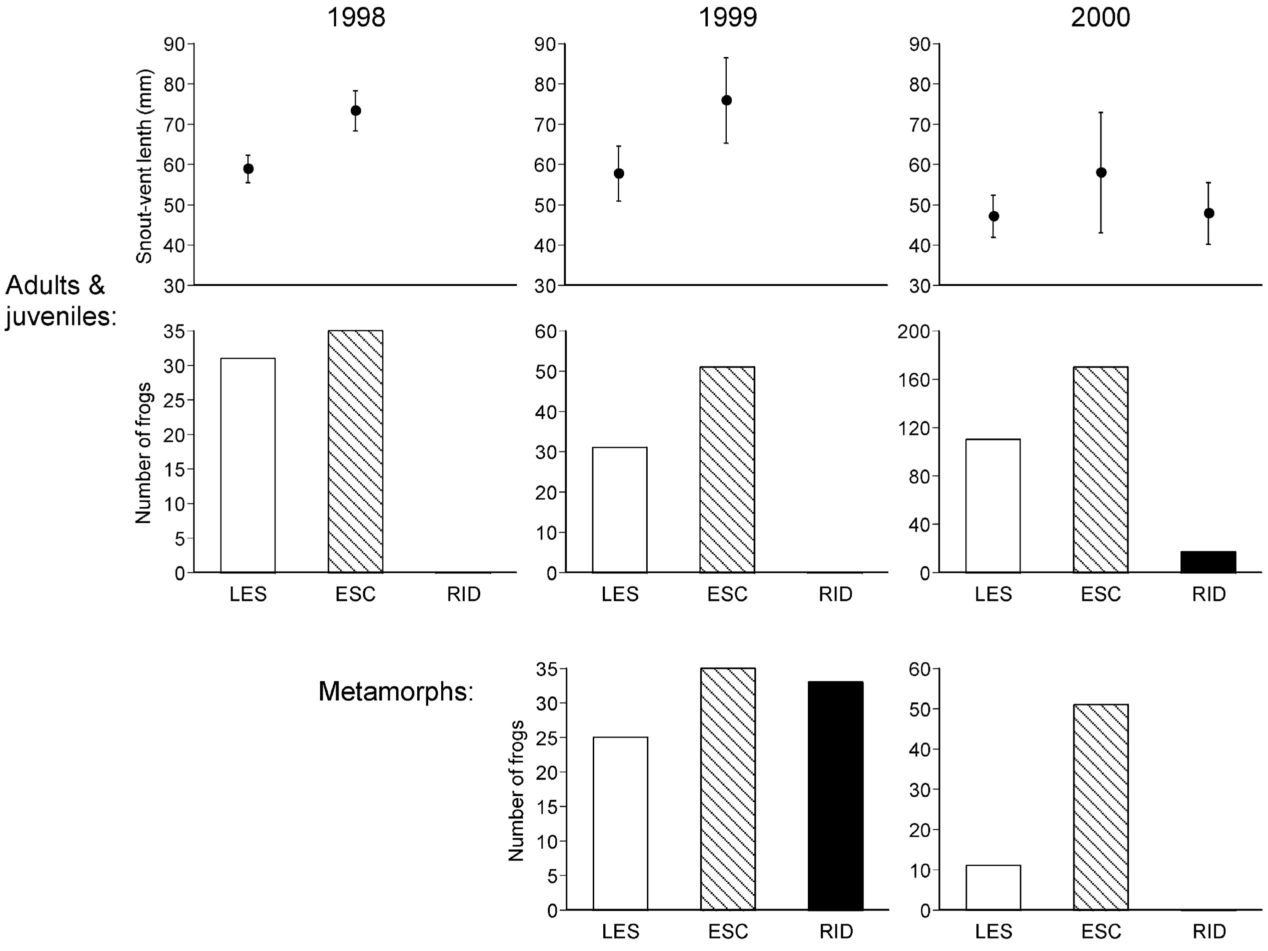

Figure 2 Taxon composition and mean (± 1 SD) snout-vent length of adult and juvenile waterfrogs captured at Elliker Auen in 1998, 1999 and 2000, and taxon composition of metamorphs captured in 1999 and 2000. LES indicates *R. lessonae*, ESC indicates *R. esculenta* and RID indicates *R. ridibunda.*

cohort seemed to have decreased compared to the time of metamorphosis. Based on data from Neveu (1991), I assumed that all *R. lessonae* below 45 mm SVL, all *R. esculenta* below 50 mm SVL, and all *R. ridibunda* captured in 2000 were one year old (total $n = 116$). Further assuming that the taxon composition of the 1999 sample of metamorphs was representative, the frequency of *R. ridibunda* decreased from 35.5% at metamorphosis to 14.7% in the following year, whereas the relative frequencies of *R. esculenta* and *R. lessonae* increased from 37.6 to 48.3% and from 26.9 to 37.0%, respectively ($\chi^2 = 12.3$, d.f. = 2, $P = 0.002$). This rough calculation probably still overestimates the relative frequency of *R. ridibunda* in the cohort, because one-year-old *R. lessonae* and *R. esculenta* can grow considerably larger than the chosen thresholds towards the end of the season (Neveu 1991); hence, some one-year-old *R. lessonae* and *R. esculenta* captured late in the season may have been neglected in the calculation, whereas all *R. ridibunda* were included.

Genetic analyses

Allele frequencies are summarized for each taxon in Table 1. Only *LDH-B* was polymorphic in *R. lessonae*. At this locus, the observed heterozygosity did not significantly deviate from that expected under Hardy–Weinberg equilibrium ($\chi^2 = 0.07$, d.f. = 1, $P = 0.79$). In *R. esculenta*, all six loci were polymorphic. Not surprisingly, there was a significant heterozygote excess in hybrids at all loci (all $P < 0.001$, except for *MPI*, where $P = 0.03$). Three loci were also variable within clonal *ridibunda* genomes in *R. esculenta*: *GPI*, *LDH-B* and *MPI*. Based on these loci, a total of five hemiclones could be distinguished (Table 2). The relative frequencies of hemiclones were similar in male and female *R. esculenta* ($\chi^2 = 5.55$, d.f. = 4, $P = 0.235$). In *R. ridibunda*, two loci were polymorphic: *LDH-B* and *MPI*. The observed heterozygosity at *LDH-B* did not significantly deviate from Hardy–Weinberg equilibrium ($\chi^2 = 0.45$, d.f. = 1, $P = 0.50$), but there was a significant heterozygote excess at *MPI* ($\chi^2 = 8.11$, d.f. = 1, $P = 0.004$), suggesting that many *R. ridibunda* originated from matings between hemiclones differing at *MPI*.

Table 1 Allele frequencies at six variable allozyme loci in *R. lessonae*, *R. ridibunda* and the hybrid *R. esculenta* from Elliker Auen. Allele designations follow the system of Hotz (1983).

Locus	Allele	*R. lessonae* ($n = 91$)	*R. esculenta* ($n = 161$)	*R. ridibunda* ($n = 49$)
sAAT	*e*		0.5000	1.0000
	g	1.0000	0.5000	
GPI	*a*		0.4720	1.0000
	d	1.0000	0.5280	
LDH-B	*a*		0.0458	0.0938
	b	0.2045	0.1307	
	c		0.4542	0.9062
	e	0.7955	0.3693	
MPI	*a*		0.1469	0.2979
	c	1.0000	0.8531	0.7021
PGM-2	*c*	1.0000	0.5000	
	d		0.5000	1.0000
PGDH	*c*	1.0000	0.5000	
	d		0.5000	1.0000

With the five hemiclones found in the population, *R. esculenta* × *R. esculenta* matings could potentially produce offspring with 15 combinations of hemiclones, representing 14 distinguishable multilocus genotypes. Their expected frequency distribution, assuming random mating and equal survival probabilities of all offspring, is illustrated in Fig. 3. Among the field-caught *R. ridibunda*, I only detected four genotypes, all of which were consistent with their being formed by matings between *R. esculenta*. Overall, the frequency distribution of observed genotypes did not significantly deviate from the expected frequencies (Fig. 3). Nevertheless, the two genotypes that arise from matings that combine hemiclone EL 1 with EL 2, EL 1 with EL 5, and EL 2 with EL 3 (the latter two combinations produce the same offspring genotype) were somewhat over-represented compared to the expectation (Fig. 3). These two genotypes are heterozygous at *MPI*, which explains the significant heterozygote excess in *R. ridibunda* at this locus.

Survival and development of metamorphs

When raised in indoor terraria, metamorphs of *R. esculenta* and *R. ridibunda* suffered from a somewhat higher mortality than *R. lessonae*: three of 11 *R. esculenta*, nine of 33 *R. ridibunda* and none of the 11 *R. lessonae* died before the first hibernation. However, the difference between taxa is not significant (Fisher's exact test, $P = 0.191$). No additional mortality occurred during the first hibernation in the cool room.

All 24 surviving *R. ridibunda* were females ($\chi^2 = 24.00$, d.f. = 1, $P < 0.001$), whereas the observed sex ratio in *R. lessonae* (four males and seven females; $\chi^2 = 0.82$, d.f. = 1, $P = 0.37$) and in *R. esculenta* (four males and four females, $\chi^2 = 0.00$, d.f. = 1, $P = 1.00$) did not significantly deviate from equality.

Because the *R. ridibunda* females died of a bacterial infection during the second hibernation, their fertility could not be assessed. Posthumous dissection of 13 individuals revealed that 12 had normally developed ovaries with large quantities of enlarged primary oocytes, whereas one female had abnormally small ovaries without any enlarged primary oocytes.

Table 2 Hemiclones found at Elliker Auen, defined by the allozyme haplotypes of *ridibunda* genomes in the hybrid *R. esculenta.*

		Locus					
Hemiclone	Frequency (%)	*sAAT*	*GPI*	*LDH-B*	*MPI*	*PGM-2*	*PGDH*
EL 1	58.2	*e*	*a*	*c*	*c*	*d*	*d*
EL 2	27.9	*e*	*a*	*c*	*a*	*d*	*d*
EL 3	6.3	*e*	*a*	*a*	*c*	*d*	*d*
EL 4	5.7	*e*	*d*	*c*	*c*	*d*	*d*
EL 5	1.9	*e*	*a*	*a*	*a*	*d*	*d*

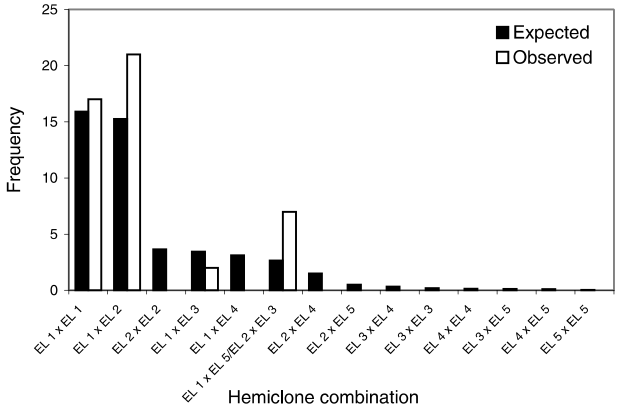

Figure 3 Expected and observed frequencies of multilocus genotypes in *R. ridibunda* produced by hybrid × hybrid matings, representing all possible combinations of hemiclones found in Elliker Auen, sorted from left to right by decreasing expected frequency. The expected frequencies were calculated under the assumption of random mating among *R. esculenta* and equal survival probabilities of all offspring. The two distributions do not differ significantly ($\chi^2 = 6.23$, d.f. = 3, $P = 0.101$). Adjacent categories were pooled for the test so that all expected frequencies ≥ 5. $n = 47$.

DISCUSSION

Several lines of evidence indicate that in the waterfrog population at Elliker Auen, non-hybrid *R. ridibunda* offspring are produced by natural matings between hemiclonal hybrid *R. esculenta*. First, experimental *R. esculenta* × *R. esculenta* crosses produced viable *R. ridibunda* tadpoles that successfully completed metamorphosis in artificial ponds (Vorburger, in press). Second, a large fraction of field-collected metamorphs were *R. ridibunda* in 1999, although no adults of *R. ridibunda* were found in the population in 1998 and 1999. Third, the multilocus genotypes of *R. ridibunda* from Elliker Auen are consistent with combinations of clonal *ridibunda* genomes found in *R. esculenta*, and fourth, these *R. ridibunda* are all females. The possibility that they rafted in from an introduced *R. ridibunda* population during the 1999 flooding can safely be ruled out, because there are no known introduced populations upstream of both rivers adjacent to the study site (Grossenbacher 1988). Furthermore, it would be highly improbable to obtain a sample consisting entirely of females belonging to no more than four different genotypes from a sexually reproducing *R. ridibunda* population.

That offspring from hybrid × hybrid matings can be viable appears to be conditional on the presence of more than one hemiclone in the population. Different clonal lineages with independent evolutionary histories are unlikely to carry the same deleterious mutations, whether they were fixed through the advance of Muller's ratchet (Charlesworth & Charlesworth 1997) or already 'frozen' at hemiclone formation. Thus, one would expect that viable *R. ridibunda* tadpoles originate from matings between rather than within hemiclones. Indeed, three of the four multilocus genotypes in *R. ridibunda* from Elliker Auen represent combinations of different hemiclones, but contrary to the expectation, matings within the most common hemiclone EL 1 also produced viable offspring. Of course, hemiclone determination using only six allozyme loci provides limited resolution. In the hybridogenetic fish *Poeciliopsis monacha-lucida*, the more sensitive technique of tissue grafting identified 18 hemiclones among 30 strains that had only been subdivided into eight hemiclones based on allozymes (Vrijenhoek *et al.* 1978; Angus & Schultz 1979). Similarly, Hotz *et al.* (2001) have shown that allozymic hemiclones in *R. esculenta* can be further subdivided using more variable microsatellite markers. Hence, some matings within hemiclone EL 1 may actually combine different clonal lineages that happen to share the same allozymic haploype. Alternatively, some clonal *ridibunda* genomes found at Elliker Auen may represent derived rather than ancient clonal lineages, if in the past *R. ridibunda* females have been formed and produced new hemiclones repeatedly (see Introduction). Such derived hemiclones may partly be freed from deleterious mutations or else have remaining mutations linked to new combinations of marker alleles, making it impossible to predict tadpole viability based on hemiclone combination.

Whatever the explanation, the hybrid population at Elliker Auen today contains some hemiclones that are genetically compatible and produce viable *R. ridibunda* offspring. So why are female *R. ridibunda* not a permanent component of this waterfrog population? Two observations point towards a possible answer to this question. First, the *R. ridibunda* metamorphs I collected in the field were produced in a year that was exceptionally favourable for tadpole development due to extremely high water levels. In the following year, when water levels were normal, the proportion of *R. ridibunda* metamorphs must have been greatly reduced, as not a single one could be found (Fig. 2). It may be that under normal conditions, *R. ridibunda* tadpoles from matings between hybrids are competitively inferior to *R. esculenta* and *R. lessonae* tadpoles, but may successfully complete metamorphosis in years of strongly reduced competition. If such years are rare (May 1999 was the rainiest May of the 20th century in northern Switzerland), *R. ridibunda* will only occur in the population sporadically. Second, *R. ridibunda* seemed to survive the first winter in the field less well than *R. lessonae* and *R. esculenta*. The reasons for this lower survival are presently unknown. I can only speculate that it may be related to the fact that *R. ridibunda* generally hibernates in the water, whereas *R. lessonae* and *R. esculenta* mainly hibernate on land (Berger 1982; Günther 1990; Holenweg & Reyer 2000). The ponds at Elliker Auen are deep enough not to freeze to the bottom in winter (they contain fish), but mortality in the water may nevertheless be higher.

Taken together, these two observations suggest that *R. ridibunda* from matings between two *R. esculenta* may be poorly adapted to the ecological conditions of the habitat in which they arise. On one hand, this is not surprising because *R. ridibunda* does not occur naturally in Switzerland. On the other hand, this is inconsistent with the fact that introduced *R. ridibunda* successfully established elsewhere in Switzerland and even replaced the native waterfrogs in some areas of the country (Grossenbacher 1988). However, those *R. ridibunda* that originally founded the Elliker Auen hemiclones through primary hybridizations may have been adapted to different environments than those *R. ridibunda* that were introduced recently.

How commonly do *R. ridibunda* arise from hybrid × hybrid matings? It is not unusual to find several hemiclones coexisting in natural L-E-systems, suggesting that this may be possible at many locations (Hotz *et al.* 1994; Semlitsch *et al.* 1997; H. Hotz, personal communication). By contrast, reports on female *R. ridibunda* found in L-E-systems are scarce. But there are reasons to believe that the frequency of this phenomenon is generally underestimated. Large-scale field surveys of anuran populations are often based on call counts (Heyer *et al.* 1994). Such surveys will not detect *R. ridibunda* in L-E-systems, because *R. ridibunda* from hybrid × hybrid matings are all females and do not call. Even when frogs are captured, *R. ridibunda* may easily be overlooked, because morphological determination of taxa in the field requires considerable experience and should be confirmed by genetic analysis. Many surveys therefore pool the three taxa as 'waterfrogs', providing no information on the possible occurrence of *R. ridibunda* in the examined populations. Clearly, more detailed surveys are needed to reveal how frequently female *R. ridibunda* are formed in natural L-E-systems.

Even if such events turn out to be truly rare, they may have important consequences if these female *R. ridibunda* are fertile and thus able to found new hemiclones. Unfortunately, this essential information for a complete test of Schmidt's (1993) model is still lacking. I lost the *R. ridibunda* females due to the outbreak of a bacterial disease and could not assess their fertility. Posthumous dissection revealed that, with one exception, these females had normally developed ovaries, but this does not prove that they are indeed fertile. Nevertheless, given the present data it cannot be excluded that some *ridibunda* genomes found in L-E-systems outside the native range of *R. ridibunda* do not have an uninterrupted history of clonal inheritance. Low rates of recombination through 'covert sex' should be sufficient to prevent the genetic deterioration of clonal genomes (Hurst *et al.* 1992; Charlesworth *et al.* 1993) and yet it is true that most studies find that hybrid × hybrid matings do not produce viable offspring in natural populations. It remains to be investigated whether this indicates that recombination between clonal genomes is absent or at least too episodic to have more obvious effects, or whether this merely reflects that the possibility for occasional recombination is probably not available to the many populations containing only a single hemiclone. Certainly, the present study urges some caution in the use of hybridogenetic waterfrogs as a model system to study the long-term effects of clonal inheritance.

ACKNOWLEDGEMENTS

I thank S. Röthlisberger for help with electrophoresis, and many volunteers for collecting frogs. H.-U. Reyer, B. R. Schmidt, J. Van Buskirk and two anonymous reviewers provided valuable comments on the manuscript. This study was supported by the Swiss National Fund (31-40688.94 to H.-U. Reyer).

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BIOSKETCH

Christoph Vorburger's research interests focus on the evolution and ecology of alternative reproductive modes. He presently studies the consequences of clonal inheritance in hybridogenetic waterfrogs of the *Rana esculenta* complex.

Editor, L. Keller
Manuscript received 5 July 2001
First decision made 8 August 2001
Manuscript accepted 7 September 2001