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Demography and evolution of pure hybridogenetic frog (*Rana esculenta*) populations

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ABSTRACT

Question: How can pure hybrid populations of the hemiclonal frog *Rana esculenta* persist over time? How can they maintain genetic diversity despite partial clonal inheritance?

Mathematical methods: A deterministic model for identifying the composition of hybrid populations in relation to gamete production and primary fitness of its diploid and triploid members. Computer simulations for testing the effects of population composition on genetic diversity.

Key model assumptions: Pure *Rana esculenta* populations consist of diploid males and females of the genotype LR and triploid males and females of the type LLR. Triploids of both sexes eliminate the R genome pre-meiotically (hybridogenesis) and produce haploid L gametes. Within the diploids, males produce R sperm and females either haploid R or diploid LR eggs. All individuals mate randomly and generations do not overlap. The overall hybrid population has a constant size with both sexes and ploidies affected equally by the limitation.

Predictions: In pure *Rana esculenta* populations, the co-existence of diploid and triploid individuals is stable since each ploidy depends on the other for successful reproduction; hence, the mating system is balanced in itself. The genetic diversity and health in these hemiclonal populations resembles the diversity in similar sexual species due to a constant high amount of recombination in one of the parental genomes and a reduced mutation rate in the other. Thus diploid–triploid *R. esculenta* have become self-sustaining evolutionary units with a potential for new species formation.

Keywords: ecological modelling, evolutionary unit, hybridogenesis, mate choice, ploidy, population dynamics, speciation.

INTRODUCTION

There is an ongoing debate about the role of polyploidy in the evolution of species (Otto and Whitton, 2000). Opinions about its importance range from having contributed little to progressive evolution (Stebbins, 1971) to being a major factor in the evolution of animal species (Schultz, 1980). Whereas in plants polyploidy is widespread (Masterson, 1994) and its importance in speciation is well recognized, polyploidy in animals is still viewed as some sort of an

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evolutionary 'mishap'. This is especially the case for triploid animals, which are often considered to be evolutionary dead ends due to their usually low fertility. This low fertility arises from problems in chromosomal pairing and segregation during meiosis caused by the odd number of genomes. But once triploid organisms have overcome these obstacles, they may very well play an active role in speciation through hybridization. In the case we will investigate in this article, triploids enable the transition from a host-bound, hybridogenetically reproducing organism to a self-propagating evolutionary unit.

Hybridogenesis (Schultz, 1969) is a rare reproductive mode, almost exclusively confined to unisexual (i.e. all-female) vertebrates. Like all unisexual vertebrates, hybridogens are of interspecific origin (Dawley, 1989). Their parental genomes do not recombine (Schultz, 1969) during gamete reproduction. Instead, one of the paternal genomes is excluded entirely and only the other unaltered parental genome is passed on to the gametes. Diploid hybridogenetic organisms thus are reproductively equivalent to one of their parental species, except that hybridogenetically transmitted genomes do not recombine. As a consequence, to persist, hybridogens have to regularly backcross with the parental species whose genome they have excluded. Schultz (1969) suggested that the novel gene combinations in hybridogens and the associated high amount of heterozygosity qualify hybridogenetic organisms as being possible origins of new species.

However, for most hybridogenetic organisms, two major obstacles constrain the ability to become self-propagating units. First, almost all hybridogens are unisexuals. And second, they are reproductively bound to one of their sexual ancestors since they have to regain their lost genome. The hybridogenetic frog *Rana esculenta* seems to have overcome both these obstacles. *Rana esculenta* (E), the edible frog, is a bisexual hybrid between the poolfrog *Rana lessonae* (L) and the lakefrog *Rana ridibunda* (R) (Berger, 1977). In most of its area of distribution, *R. esculenta* populations consist of diploid males and females that eliminate the parental L genome before meiosis (Graf and Müller, 1979; Uzzell *et al.*, 1980) and only transmit a clonally inherited R genome. Consequently, they have to live in sympatry with *R. lessonae* to regain the lost genome.

It has been reported, however, that *R. esculenta* can sometimes exist in pure hybrid populations (e.g. Günther, 1975; Berger, 1988; Günther and Plötner, 1990; Rybacki, 1994; Mikulicek and Kotlik, 1999; Christiansen *et al.*, 2005). In most of these populations, *R. esculenta* occur as diploids and triploids (Table 1). Although triploids can occur in two forms (LLR and LRR), the most common form – and hence the one addessed in this paper – appears to be the one with two L genomes and one R genome. Crossing experiments show that the rarer genome is excluded pre-meiotically and the one present in two copies undergoes normal recombination (Berger, 1988; Günther and Plötner, 1990; M. Arioli and C. Jakob, unpublished data). Hence, gametes from LLR triploids contain a single L genome. Triploid *R. esculenta* have therefore taken over the role of the parental species *R. lessonae* by providing the diploid conspecifics with the L genome needed for persistence. Thus, mixed diploid–triploid E populations show all the preconditions necessary for evolving into a separate species in the sense of Schultz (1969): they feature both sexes and can reproduce independently of both parental species. This gives them a chance to eventually become reproductively isolated from both sexual ancestors. But the long persistence of such mixed diploid–triploid E systems is only possible if both ploidies are always present in the population.

According to Table 1, diploid LR can only persist when triploids provide them with an L genome. Triploid LLR, on the other hand, can only arise from diploid eggs of diploid females that have been fertilized with L sperm from a triploid male. Such a tight coupling of

Table 1. Ploidy, gamete types, and offspring arising from all possible mating combinations in the most common type of pure *Rana esculenta* populations

Males		LLR (Tm)	LR (Dm)
Females	Gametes	$L_{f,m}$	R_f
LLR (Tf)	L_f	$LL_{f,m}$	LR_f
LR (Df)	R_f	$LR_{f,m}$	RR_f
	LR_f	$LLR_{f,m}$	LRR_f

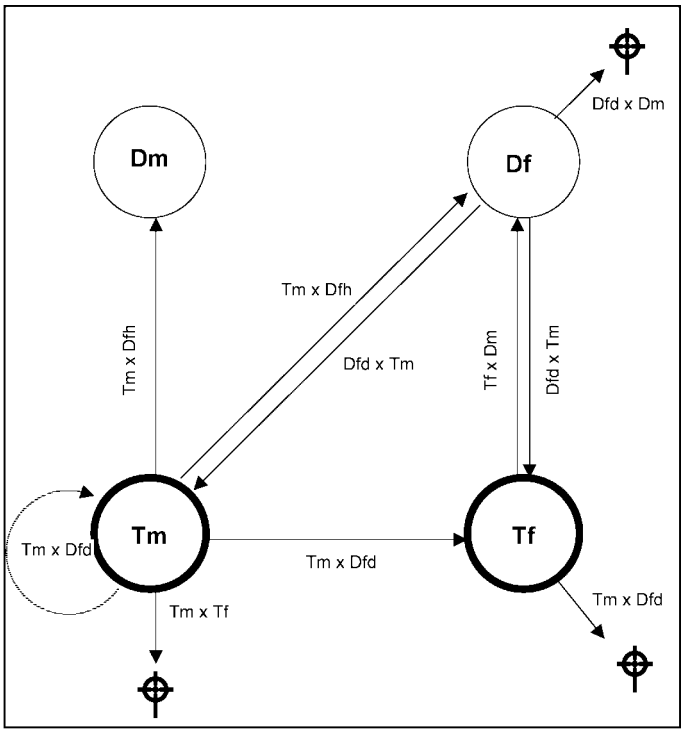
Note: The top header row for males and the left header column for females indicate the ploidy and genetic composition. Terms in brackets are the abbreviations used throughout the text. The bottom header row for males and the right header column for females indicate the type of gametes they produce. Subscripts indicate the sex of the offspring (m = males, f = females) or whether gametes carry an X or Y chromosome. Offspring types on grey background are generally inviable.

the two ploidies raises the question of how this forced co-existence is regulated and how the diploid–triploid population composition depends on the reproductive performance of the different types. Furthermore, the population composition has implications for the evolutionary properties of such a diploid–triploid system. L genomes can only recombine in triploid individuals, whereas diploid gametes of diploid *R. esculenta* contain unaltered, clonally transmitted L and R genomes. The diploid–triploid ratio in pure E populations may therefore have a direct influence on the frequency of recombination of L genomes over time. Recombination is particularly important in populations of a limited size, since stochastic drift effects can lead to a substantial accumulation of deleterious mutations or to the loss of allele diversity if genomes are mostly clonally transferred (Haig, 1978; Pamilo, *et al.*, 1987; Charlesworth *et al.*, 1993; Charlesworth and Charlesworth, 1997; Rice, 1998). The long-term success of diploid–triploid E populations thus not only depends on the constant presence of both ploidies but also on how well size-limited populations cope with the problems of drift and mutation accumulation that arise from the partly clonal inheritances. Unfortunately, the mutagenic properties of polyploid animals are largely unknown and we have to limit this study to the investigation of allele diversity in limited pure E populations.

Inheritance pathways of L and R alleles

In mixed diploid–triploid E populations, L and R alleles can be passed on from diploid to triploid organisms, from males to females, and vice versa. In triploid organisms, two haploid L genomes are paired that may have been clonally transferred previously, namely when they have come from diploid eggs. In such triploid *R. esculenta*, recombination of the L genome is likely to occur, which contributes significantly to the genetical health of a pure E population. But apart from the possibility for recombination, certain mating combinations can lead to dead ends for L or R alleles. L alleles in diploid LR males or R alleles in triploid LLR, for example, will not be transferred to the next generation (see Table 1). Figures 1a

a)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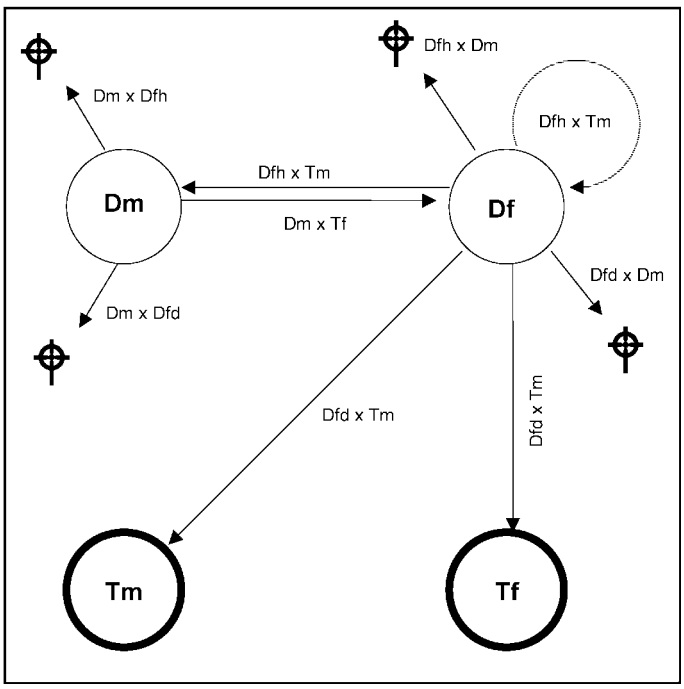
b)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and 1b show the different inheritance pathways L and R alleles can take in pure *R. esculenta* populations.

The frequency of recombination of L genomes, which largely influences the population genetical properties of pure E populations, cannot be easily predicted from the inheritance pathways in Fig. 1a. Furthermore, the frequency of transitions between the different sexes and ploidies may depend on the population composition. R genomes do not recombine in diploid–triploid populations of the LR–LLR type but, as can be seen in Fig. 1b, R alleles from diploid males that are passed on to the next generation will always end up in daughters, whereas R alleles from diploid females can be transferred to male and female offspring.

This means that R alleles will spend on average more time in females than in males. If mutation rates in diploid *R. esculenta* are sex biased, this will affect the overall mutagenesis an R allele has been subjected to (Som and Reyer, 2006). Again, Fig. 1b alone does not allow a straightforward prediction of this sex-biased mutagenesis and the magnitude of the effect may vary with the actual population composition.

The aim of this article is to examine whether diploid–triploid E populations can persist over time and how the forced co-existence of diploids and triploids is regulated. We do this by developing a deterministic model that allows identification of the population composition of diploid–triploid E populations, in relation to the frequency of haploid versus diploid egg production of diploid E females and to the differences in primary fitness of diploid versus triploid females. Furthermore, we present the results of computer simulations that reproduce the effect of population composition on the recombination frequency of L genomes, the evolutionary speed of R genomes, and the allele diversity in limited E populations.

MATHEMATICAL METHODS

Population composition

For the deterministic model of a pure *R. esculenta* population, we make the following eight assumptions:

1. The outcomes of mating combinations and the type of gametes produced by the involved individuals follow Table 1.
2. All individuals mate randomly. A study by Günther and Plötner (1990) revealed no evidence for assortative mating with regard to male size or type.
3. Diploid E females produce haploid eggs with an average frequency of a and diploid eggs with an average frequency of $1 - a$.
4. The diploid–triploid female fertilities show the ratio $1 : b$.

Fig. 1. Inheritance pathways of sex-unlinked L alleles (a) and R alleleles (b) in diploid–triploid *R. esculenta* populations. Arrows indicate the propagation of an L allele with its source of origination and its destination. Labels next to the arrows indicate the mating combinations that lead to the respective transition. Dm = diploid male, Dfh = haploid egg from a diploid female, Dfd = diploid egg from a diploid female, Tm = triploid male, Tf = triploid female. Crosses indicate mating combinations where L alleles would end in inviable offspring. Bold circles around Tm and Tf indicate the triploid nature and the capability of recombination in these types.

5. The overall E population has a constant size K and is limited to 1000 individuals with both sexes and ploidies affected equally by the population limitation.
6. Generations are not overlapping.
7. Triploid males produce offspring with an even sex ratio.
8. All females mate once, whereas males can mate several times.

According to Table 1, diploid males (Dm) of the next generation can only arise from triploid male (Tm) sperm fertilizing haploid eggs from diploid females (Df). Thus

$$\mathrm{D'm}(t+1) = a\mathrm{Df}(t)\,\frac{\mathrm{Tm}(t)}{\mathrm{Tm}(t)+\mathrm{Dm}(t)} \tag{1}$$

Here, $\mathrm{D'm}(t+1)$ refers to the number of juvenile diploid males before the overall population reduction to size K has occurred.

Diploid females arise from the sperm of diploid males fertilizing eggs from triploid females or from the sperm of triploid males fertilizing haploid eggs from diploid females. Therefore

$$\mathrm{D'f}(t+1) = 2b\mathrm{Tf}(t)\,\frac{\mathrm{Dm}(t)}{\mathrm{Tm}(t)+\mathrm{Dm}(t)} + a\mathrm{Df}(t)\,\frac{\mathrm{Tm}(t)}{\mathrm{Tm}(t)+\mathrm{Df}(t)} \tag{2}$$

The first term of equation (2) is multiplied by 2 because diploid males induce all female offspring (cf. Table 1). Thus, everything else being equal, twice as many female offspring arise from the first mating combination than from the second. Triploid females (Tf) and males are only produced by one mating combination: the sperm of triploid males fertilizing diploid eggs from diploid females. As triploid male offspring show an even sex ratio,

$$\mathrm{T'f}(t+1) = \mathrm{T'm}(t+1) = (1-a)\mathrm{Df}(t)\,\frac{\mathrm{Tm}(t)}{\mathrm{Tm}(t)+\mathrm{Dm}(t)} \tag{3}$$

Because the juvenile population is now reduced to the maximum sustainable population size,

$$\mathrm{Dm}(t+1) = K^{*}\,\frac{\mathrm{D'm}(t+1)}{\mathrm{D'm}(t+1)+\mathrm{D'f}(t+1)+\mathrm{T'm}(t+1)+\mathrm{T'f}(t+1)}$$

Df, Tm, and Tf are calculated accordingly.

To calculate the development of a pure E population with a given starting composition Dm(0), Df(0), Tm(0), and Tf(0), equations (1), (2), and (3) can now be iterated over the desired number of generations.

Recombination frequencies of L genomes and the mutation rate of R genomes

The easiest way to investigate the mechanisms mentioned in the Introduction is by following the fate of individual alleles at one locus from generation to generation. By recording the frequency of how often L alleles have spent their evolutionary time in recombining triploids or how often an R allele has been in a female, we can estimate the frequency of recombination or the average evolutionary rate of R genomes.

Such an assessment can be done by a non-deterministic, probabilistic simulation. For this simulation, we make assumptions 1–5 as for the model plus the following three:

- At the locus of investigation, all individuals carry an R allele plus one L allele in diploids and two L alleles in triploids.
- The alleles are not sex linked and in triploids recombination operates in a Mendelian way – that is, both L alleles have the same chance of being transferred further on.
- In the starting population, all alleles are different from each other.

The population development follows the same sequence from generation to generation: First, a male and female are picked randomly from the pool of individuals. As laid out in the model conditions, female fecundities differ between the ploidies. We account for that by giving the female type with a lower fecundity a lesser chance of reproduction. If a female does not reproduce successfully, both the male and female are put back into the pool and selection starts anew. If reproduction takes place, the next procedure depends on the male and female types involved. If the female is diploid, it produces a haploid egg with probability a of containing the mother's R allele, otherwise it produces a diploid egg containing the mother's R and L alleles. If the male is triploid, it produces a male or a female inducing sperm with the same probability and a random one of its two L alleles will be present in the single offspring. If the male is diploid, mating with a diploid female produces inviable offspring and the procedure is stopped. If the chosen female is triploid, it produces a haploid egg containing a random one of its two L alleles. If the male it mates with is diploid, the sex of the single offspring automatically becomes female and the daughter carries the R allele of its father. If the male involved is triploid, the triploid–triploid mating produces inviable offspring and the procedure is stopped. In all instances, males and females are put back into the pool regardless of whether reproduction was successful or not.

The whole process of selecting the mating partners and determining the outcome of the mating is then repeated until a new generation of 1000 individuals has been built. The new population then replaces the old population in the next generation. After 1000 generations, we recorded how many different alleles were left, how often they had been in a recombining individual, and how much of their history R alleles had spent in a female.

Since this simulation is probabilistic, we repeated it ten times for each parameter combination of a and b and calculated the averages of the different results. We then compared the average results of the allele frequency test in E populations with the results from a separate simulation of a normal sexual population of the same size.

RESULTS

Deterministic population structure model

With our deterministic model, diploid–triploid *Rana esculenta* populations of the LR–LLR type evolve into a stable equilibrium under all combinations of a and b as long as a is different from 0.0 and 1.0. We did not find any oscillation in any of the parameter combinations used. The final stable population structure is usually attained within a few generations and the population composition does not depend on the starting conditions as long as all types are present at the beginning.

Figure 2 shows the population composition after 1000 generations for different combinations of gamete ploidy frequencies of diploid females and differences in primary fitness of diploid and triploid females. Increasing the fertility of triploid females versus

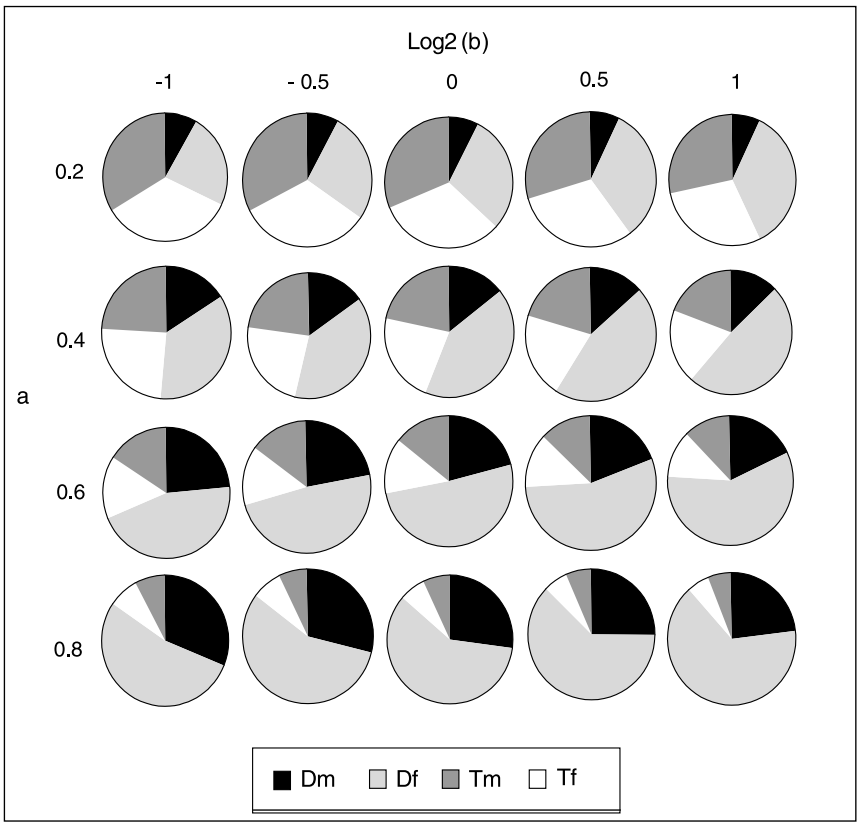

Fig. 2. Population structure of a large diploid–triploid *R. esculenta* population after 1000 generations, in relation to diploid female gamete production and differences in primary fitness of diploid versus triploid females. *a* indicates the average proportion of haploid gametes among all gametes produced by diploid females (if $a = 0.2$, 20% of a diploid female's gametes are haploid, 80% diploid). *b* indicates the relative fertility of triploid females compared with diploid females (if $b = 0.5$ or $\log2(b) = -1$, triploid females produce half the offspring that diploid females do). Dm = diploid males, Df = diploid females, Tm = triploid males, Tf = triploid females.

diploid females (i.e. increasing *b*) surprisingly increases the abundance of diploids in the population. This is mainly due to the production of all-female diploids which further increases the female bias that exists in the population anyway. If we pool ploidies over sexes, the increase in diploid females more than compensates for the reduction in triploid females and the combined female frequency increases as well with increasing *b*.

If diploid females increase their production of haploid gametes (i.e. increasing *a*), diploids of both sexes increase their abundance as well, with the greater shift in the diploid–triploid ratio occurring in males. Whereas triploid males are much more abundant than diploid males in populations with a low *a*, the ratio is reversed if diploid females produce mainly haploid eggs (high *a*). Two general patterns can be observed with increasing *a* and *b*. First, if we pool over ploidies, the sex ratio becomes more female biased, ranging from 58% females for $a = 0.2$ and $\log2(b) = -1$ to 71% females for $a = 0.8$ and $\log2(b) = 1$. Second,

when pooling over sexes, the percentage of diploids in the population ranges from 32% for $a = 0.2$ and $\log2(b) = -1$ to 88% diploids for $a = 0.8$ and $\log2(b) = 1$.

Recombination frequencies and evolutionary rate of the R genome

The tracking of individual L alleles during a 1000-generation simulation showed that L alleles spend on average two out of three generations in recombining triploid *R. esculenta*. Surprisingly, this frequency is totally independent of egg ploidy (a) and fertility (b) and thus the actual population composition. Even if the total population consists of only 32% diploids ($a = 0.2$, $\log2(b) = -1$; see Fig. 2), the surviving L alleles, which have successfully avoided all 'pitfall traps' of exclusion through matings with the wrong types or losses through drift during 1000 generations, have spent two-thirds of their evolutionary history in triploids. L genomes 'trapped' in diploid–triploid *R. esculenta* populations therefore undergo recombination quite frequently.

Contrary to the evolutionary history of L genomes, the clonal R genome evolution in pure E populations is affected by the average ploidy of eggs from diploid E females (a) and the difference in primary fitness of the two female types (b). For all parameter combinations of a and b, the clonally transmitted R genomes spend more time in females than in males (between 56 and 71% of the total simulation generations). This reduces the overall evolutionary rate of the R genomes, compared with a sexual population (Redfield. 1994), if there exists a sex bias in the mutation rate with males having a higher mutation rate than females (Hurst and Ellegren, 1998; McVean, 2000).

The amount of the reduction of the overall evolutionary rate depends on the difference in the male and female mutation rates. The greater the difference, the greater is the reduction. Figure 3 shows the amount of reduction if males have a ten times higher mutation rate than females, a figure that has been found for humans (Montandon *et al.*, 1992) and which is probably a conservative estimate for frogs, since male-to-female mutation rates are supposed to be high in externally fertilizing species (Redfield, 1994). The evolutionary rate of R genomes decreases with increasing proportions of haploid eggs (a) but also with decreasing fertility of triploids (b). This is quite remarkable since, according to Fig. 1b, R genomes can only migrate repeatedly between diploid males and diploid females and diploid females are most common if b is high. Thus a condition increasing the frequency of diploid females (high b) decreases the amount of evolutionary time that R genomes stay in diploid females. The reduction of evolutionary speed ranges from 92% ($a = 0.2$, $\log2(b) = 1$) of the speed of a normal sexual population to 66% ($a = 0.8$, $\log2(b) = -1$), always under the condition that males have a ten times higher mutation rate than females.

Allele diversity in limited pure E populations

Since we followed the fate of individual alleles at one locus, we were able to determine the number of different alleles that were left in a population of 1000 *R. esculenta* after 1000 generations of population development. To obtain a reference value, we let a sexual population of the same size evolve over the same number of generations. At the beginning of the simulation, all *R. esculenta* individuals had a different R allele and one L allele in diploids and two L alleles in triploids. The sexual population featured two different alleles per locus and individual. After 1000 generations, an average of 4.3 alleles ($n = 10$) from the initial 2000 were left in the sexual population. Diploid–triploid populations started with

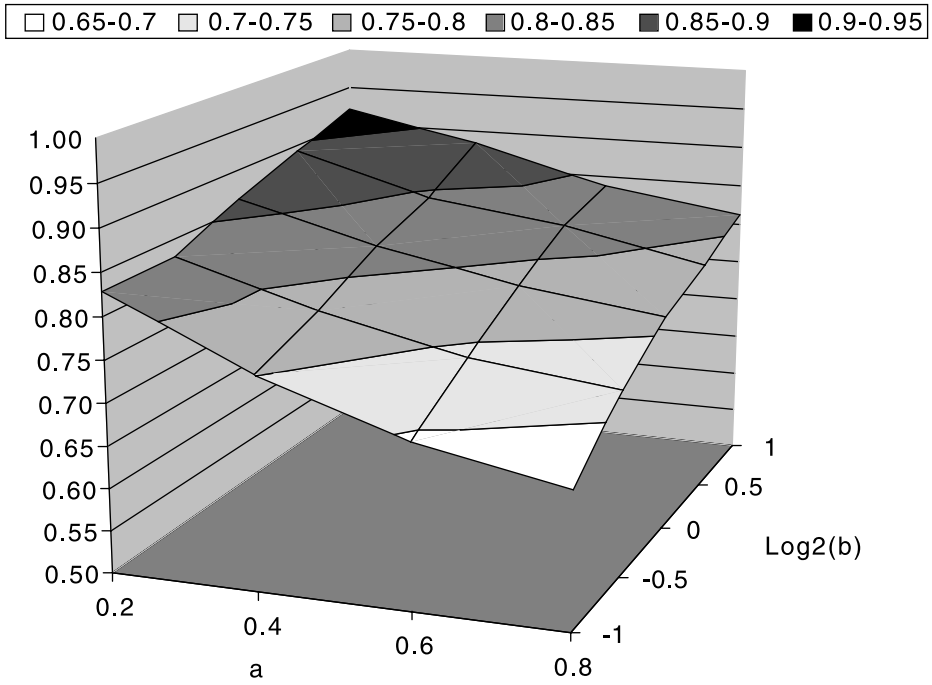

Fig. 3. Relative reduction of the speed of mutagenesis in R genomes of pure E populations compared with the mutagenetic speed of sexual populations if males have a 10 times higher mutation rate than females. The amount of reduction depends on the size of *a* and *b*.

1500 L alleles (250 individuals per sex and ploidy) and 1000 R alleles. After 1000 generations, between 1.1 and 3.4 different L alleles were left in the population and between 1.1 and 2.5 R alleles (Table 2, $n = 10$).

The combined diversity at the locus ranges from 3.0 to 4.7. The diversity of L alleles decreases with an increasing proportion of haploid eggs from diploid females (increasing *a*), since this reduces the number of triploids in the population who can hold two L alleles per locus. At the same time, the number of R alleles increases, as only diploids propagate R alleles successfully (see Table 1). The combined allelic diversity (3.0 to 4.7 different alleles per locus) lies well within the range of a comparable sexual population (4.3 different alleles per locus).

DISCUSSION

Population structure

The results from the deterministic population model show that co-existence of diploid and triploid *R. esculenta* is possible under a wide range of different female fertility types and different ratios of haploid versus diploid egg production in diploid females. For diploid mixed *R. lessonae*/*R. esculenta* (LE) populations, theoretical models predict that with a fixed population size *K*, random mating easily leads to an overshoot-collapse pattern, and assortative mating is a prerequisite for the long-term co-existence between the hybrid and its

Table 2. Allele diversity at one sex-unlinked locus in a limited diploid–triplod *Rana esculenta* population of 1000 individuals after 1000 generations for various values of *a* and *b*

a	log2(*b*)	−1		−0.5		0		0.5		1	
0.2	L alleles	3.0	4.3	2.8	3.9	3.4	4.7	2.5	3.7	3.1	4.2
	R alleles	1.3		1.1		1.3		1.2		1.1	
0.4	L alleles	2.6	4.0	2.7	4.5	2.3	4.0	2.9	4.2	3.0	4.6
	R alleles	1.4		1.8		1.7		1.3		1.6	
0.6	L alleles	2.0	4.2	2.0	3.6	2.1	4.0	2.0	3.6	1.1	3.6
	R alleles	2.2		1.6		1.9		1.6		2.5	
0.8	L alleles	1.1	3.6	1.5	3.3	1.7	3.5	1.5	3.5	1.5	3.0
	R alleles	2.5		1.8		1.8		2.0		1.3	

Note: For each combination of *a* and *b*, the left-hand entries show the number of L alleles in the population at the investigated locus (top) and the number of R alleles (bottom). The right-hand entries show the combined (L + R) diversity. All values in the table are the averages of 10 runs for each parameter set of *a* and *b*. Starting populations consisted of 250 individuals per sex and ploidy. Allele diversity in a comparable sexual population is 4.3.

sexual parental species *R. lessonae* (Som *et al.*, 2000; Hellriegel and Reyer, 2000; Reyer *et al.*, 2004). In these mixed populations, mate choice does indeed occur in females but not in males (Abt and Reyer, 1993; Roesli and Reyer, 2000; Engeler and Reyer, 2001; Schmeller *et al.*, 2005). In contrast, diploid–triploid pure *R. esculenta* populations remain stable when individuals mate randomly. Table 1 shows that all E types can only reproduce successfully when they mate with a partner of a different ploidy. It is thus inherent in the mating system that none of the ploidies can completely replace the other. Furthermore, it can easily be verified from Table 1 that a stochastic increase in the frequency of diploid females in one year would result in a higher proportion of triploid females in the offspring and vice versa.

These relationships might be the reason why Günther and Plötner (1990) found no evidence for assortative mating in *R. esculenta* from pure hybrid populations. Although Table 1 seems to suggest that it would be advantageous, at least for females, to avoid matings not leading to viable offspring, the inheritance pathways of this system (Fig. 1) are such that a selection mechanism against non-fertile matings can hardly evolve. Let us assume that a mutation on the successfully propagated L gamete of a triploid female (Tf) would lead to a preference for diploid males (Dm). A Tf × Dm mating would then obligatorily produce a diploid daughter with a preference for diploid males, which is the wrong ploidy preference for a diploid female. Successfully reproducing diploid females, on the other hand, produce both diploid daughters (Df) that should choose Tm males and triploid daughters (Tf) that should choose Dm males. A preference for a certain male ploidy would thus always be detrimental to the inclusive fitness of one of the daughter strands.

The overall results of the population composition model correspond well with the available field data (e.g. Günther, 1975; Rybacki, 1994): the mixed populations are typically female-biased with a marked female excess in diploids and a more even sex ratio in triploids The diploid–triploid ratio is very variable (ranging from about 20 : 1 to 1 : 9) and depends on both the proportion of haploid versus diploid eggs from diploid females and the difference in female type primary fitness. Naturally occurring populations with a strong triploid male bias (Ogielska *et al.*, 1994) cannot evolve in our model and have to be considered as sink populations.

Recombination frequencies and evolutionary rate of the R genome

The result that the recombination frequency of L genomes is absolutely independent of the diploid–triploid ratio in the population may be surprising at first glance. But Fig. 1 demonstrates that all L alleles present in diploid females will show up in a triploid individual if they survive to the next generation. On the other hand, all surviving L alleles from triploid females will be found in diploid females in the next generation. All matings that allow triploid males to transfer an L allele are matings with diploid females. The probability of which path an L allele takes from a triploid male is thus independent of the diploid–triploid female ratio. All the allele transfers described above are independent of the diploid–triploid ratio; rather, they are determined by the smallest group of allele donors or receivers.

Since we have no reason to assume that recombinations between the two L genomes in a triploid *R. esculenta* differ qualitatively from the recombinations in pure *R. lessonae* individuals, the constant relatively high amount of recombinations should allow the L genomes in pure E populations to remain largely intact – especially when considering that several researchers have suggested that most benefits of sex or recombination already accrue if only a small part of otherwise asexual organisms reproduce sexually (Charlesworth *et al.*, 1993; Green and Noakes, 1995; Peck *et al.*, 1997; but see also Peck and Waxman, 2000).

Furthermore, the genetic diversity of pure E populations is ensured through a second interesting mechanism. By comparing the inheritance pathways from Figs. 1 and 2, one can see that it is impossible that pairs of L and R alleles 'travel' together in time through the population. Whereas persisting R alleles obligatorily have to migrate between diploid males and females or to stay in diploid females, successful L alleles cannot do either of these two things. The L and R alleles at one locus are thus reshuffled every generation anew in all individuals of all types.

In contrast to the L genomes, the R genomes in pure E populations cannot profit from the benefits of recombination. Due to their strictly clonal inheritance, they are likely to suffer from Muller's ratchet (Muller, 1964; Charlesworth and Charlesworth, 1997). But since the overall mutation rate and thus the rate of the occurrence of deleterious mutations is lower in the R genomes of hybrid populations than the deleterious mutation rate of sexual populations, the ratchet may be slowed down sufficiently to prevent a substantial deleterious mutation load (Som and Reyer, 2006).

Allele frequencies

Our simulation result, that the allele diversity in a limited pure hybrid population does not differ significantly from the allele diversity of a sexual population of similar size, confirms the results of other studies which showed that the genetic diversity of polyploids is often similar or higher than the diversity in related diploids (Kobel and Du Pasquier, 1986; Brochmann *et al.*, 1992; Soltis and Soltis, 1993). It would appear that the higher number of alleles in a partly polyploid E population compared with a sexual diploid population (Otto and Whitton, 2000) can compensate for the loss of variability through drift effects on the clonally transmitted R genome. Furthermore, if we assume that L and R alleles are principally different, the minimum number of different alleles per locus can never be lower than 2. Since the rate of evolution of specific traits, which is a prerequisite for speciation, does not only depend on the selection on traits but also on their genetic variability (Fisher, 1930), pure E populations should

have the same evolutionary potential as comparable sexual populations. All the findings mentioned above suggest that *R. esculenta* in diploid–triploid populations have successfully taken the step from host-bound sexual parasites to independent, self-sustaining evolutionary units. If triploid animals really are an evolutionary mishap, in this instance it has been a very fortunate one.

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REFERENCES

Abt, G. and Reyer, H.-U. 1993. Mate choice and fitness in a hybrid frog: *Rana esculenta* females prefer *Rana lessonae* males over their own. *Behav. Ecol. Sociobiol.*, **32**: 221–228.

Berger, L. 1977. Systematics and hybridization in the *Rana esculenta* complex. In *The Reproductive Biology of Amphibians* (D.H. Taylor and S.I. Guttmann, eds.), pp. 367–388. New York: Plenum Press.

Berger, L. 1988. An all-hybrid water frog population persisting in agrocenoses of central Poland (Amphibia, Salientia, Ranidae). *Proc. Natl. Acad. Sci. Philadelphia*, **140**: 202–219.

Brochmann, C., Soltis, D.E. and Soltis, P.S. 1992. Electrophoretic relationships and phylogeny of nordic polyploids in *Draba (Bracssicaceae). Plant Syst. Evol.*, **182**: 35–70.

Charlesworth, B. and Charlesworth, D. 1997. Rapid fixation of deleterious alleles can be caused by Muller's ratchet. *Genet. Res.*, **70**: 63–73.

Charlesworth, D., Morgan, M.T. and Charlesworth, B. 1993. Mutation accumulation in finite outbreeding and inbreeding populations. *Genet. Res.*, **61**: 39–56.

Christiansen, D.G., Fog, K., Pedersen, B.V. and Boomsma, J.J. 2005. Reproduction and hybrid load in all-hybrid populations of *Rana esculenta* water frogs in Denmark. *Evolution*, **59**: 1348–1361.

Dawley, R.M. 1989. An introduction to unisexual vertebrates. In *Evolution and Ecology of Unisexual Vertebrates* (R.M. Dawley and J.P. Bogart, eds.), pp. 1–18. Albany, NY: The New York State Museum, Bulletin #466.

Engeler, B. and Reyer, H.-U. 2001. Choosy females and indifferent males: mate choice in mixed populations of the water frogs *Rana lessonae* and *Rana esculenta. Behav. Ecol.*, **12**: 600–606.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press.

Graf, J.-D. and Müller, W.P. 1979. Experimental gynogenesis provides evidence of hybridogenetic reproduction in the *Rana esculenta* complex. *Experientia*, **35**: 1574–1576.

Green, R.F. and Noakes, D.L.G. 1995. Is a little bit of sex as good as a lot? *J. Theor. Biol.*, **174**: 87–96.

Günther, R. 1975. Zum natürlichen Vorkommen und zur Morphologie triploider Teichfrösche '*Rana esculenta*' (L.) in der DDR (Anura, Ranidae). *Mitt. Zool. Mus. Berlin*, **51**: 145–158.

Günther, R. and Plötner, J. 1990. Mating pattern in pure hybrid populations of water frogs, *Rana* kl. *esculenta* (Anura, Ranidae). *Alytes*, **8**: 90–98.

Haig, J. 1978. The accumulation of deleterious genes in a population – Muller's Ratchet. *Theor. Popul. Biol.*, **14**: 251–267.

Hellriegel, B. and Reyer, H.-U. 2000. Factors influencing the composition of mixed populations of a hemiclonal hybrid and its sexual host. *J. Evol. Biol.*, **13**: 906–918

Hurst, L.D. and Ellegren, H. 1998. Sex biases in the mutation rate. *Trends Genet.*, **14**: 446–452.

Kobel, H.R. and Du Pasquier, L. 1986. Genetics of polyploid *Xenopus. Trends Genet.*, **2**: 310–315.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in the majority of angiosperms. *Science*, **264**: 421–423.

McVean, G. 2000. Evolutionary genetics: What is driving male mutation? *Curr. Biol.*, **10**: R834–R835.

Mikulicek, P. and Kotlik, P. 1999. Pure hybrid populations (*Rana esculenta*) in Western Slovakia. Paper presented at the *3rd International Symposium on Western Palearctic Water Frogs*, Berlin, Germany, October.

Montandon, A.J., Green, P.M., Bentley, D.R. Ljung, R., Kling, S., Nilsson, I.M. *et al.* 1992. Direct estimate of the Hemophilia-B (factor-IX deficiency) mutation-rate and of the ratio of the sex-specific mutation-rates in Sweden. *Human Genet.*, **89**: 319–322.

Muller, H.J. 1964. The relation of recombination to mutational advance. *Mutation Res.*, **1**: 2–9.

Ogielska, M., Kubicius, I., Gruszka, K., Odziomek, B., Poreba, M., Indyk, M. *et al.* 1994. Diploid–triploid, predominantly pure *Rana esculenta* population in Wroclaw-Nowy Dom. *Zool. Poloniae*, **39**: 501–502.

Otto, S.P. and Whitton, J. 2000. Polyploid incidence and evolution. *Annu. Rev. Genet.*, **34**: 401–437.

Pamilo, P., Nei, M. and Li, W.-H. 1987. Accumulation of mutations in sexual and asexual populations. *Genet. Res.*, **49**: 135–146.

Peck, J.R. and Waxman, D. 2000. What's wrong with a little sex? *J. Evol. Biol.*, **13**: 63–69.

Peck, J.R., Barreau, G. and Heath, S.C. 1997. Imperfect genes, Fisherian mutation and the evolution of sex. *Genetics*, **145**: 1171–1199.

Redfield, R.J. 1994. Male mutation rates and the cost of sex for females. *Nature*, **369**: 145–147.

Reyer, H.-U., Wälti, M.-O., Bättig, I., Altwegg, R. and Hellriegel, B. 2004. Low proportions of reproducing hemiclonal females increase the stability of a sexual parasite–host system (*Rana esculenta, R. lessonae*). *J. Anim. Ecol.*, **73**: 1089–1101.

Roesli, M. and Reyer, H.-U. 2000. Male vocalization and female choice in the hybridogenetic *Rana lessonae*/*Rana esculenta* complex. *Anim. Behav.*, **60**: 745–755.

Rice, W.R. 1998. Requisite mutational load, pathway epistasis, and deterministic mutation accumulation in sexual versus asexual populations. *Genetica*, **102/103**: 71–81.

Rybacki, M. 1994. Pure populations of a hybrid *Rana esculenta* from the German–Polish Usedom island. *Zool. Poloniae*, **39**: 519–520.

Schmeller, D.S., O'Hara, R. and Kokko, H. 2005. Male adaptive stupidity: male mating pattern in hybridogenetic frogs. *Evol. Ecol. Res.*, **7**: 1039–1050.

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

Schultz, R.J. 1980. Role of ploidy in the evolution of fishes. In *Polyploidy: Biological Relevance* (W.H. Lewis, ed.), pp. 313–340. New York: Plenum Press.

Soltis, D.E. and Soltis, P.S. 1993. Molecular data and the dynamic nature of polyploidy. *Crit. Rev. Plant Sci.*, **12**: 243–273.

Som, C. and Reyer, H.-U. 2006. Variation in the evolutionary rate of hemiclonal *Rana esculenta* populations. *Evol. Ecol.*, **20**: 159–172.

Som, C., Anholt, B.R. and Reyer, H.-U. 2000. The effect of assortative mating on the coexistence of a hybridogenetic waterfrog and its sexual host. *Am. Nat.*, **156**: 34–46.

Stebbins, G.L. 1971. *Processes of Organic Evolution*. Englewood Cliffs, NJ: Prentice-Hall.

Uzzell, T., Hotz, H. and Berger, L. 1980. Genome exclusion in gametogenesis by an interspecific *Rana* hybrid: evidence from electrophoresis of individual oocytes. *J. Exp. Zool.*, **214**: 251–259.