doi: 10.1111/j.1420-9101.2006.01243.x

Hemiclonal reproduction slows down the speed of Muller's ratchet in the hybridogenetic frog *Rana esculenta*

C. SOM & H.-U. REYER

Institute of Zoology, University of Zurich, Zurich, Switzerland

Keywords:

cyclical parthenogenesis;
hybridogenesis;
mutation accumulation;
Rana esculenta;
Rana lessonae;
recombination.

Abstract

Rare recombination in otherwise asexually reproducing organisms is known to beneficially influence the fitness in small populations. In most of the investigated organisms, asexual and rare sexual generations with recombination follow each other sequentially. Here we present a case where clonal reproduction and rare recombination occur simultaneously in the same population. The hybridogenetic water frog *Rana esculenta* (E), a hybrid between *R. lessonae* (L) and *R. ridibunda* (R) produces gametes that only contain the unaltered maternal R part of their genome. New generations of *R. esculenta* usually arise from E × L matings. Intraspecific E × E matings produce mostly inviable offspring, but in rare cases, female *R. ridibunda* arise from such matings which are capable of recombination. In the absence of conspecific males, these R females have to mate with E males, which results in further R females, or with L males, which produces new E lineages. This indirect mechanism reintroduces recombination into the otherwise clonally transmitted R genomes in *R. esculenta* populations. In this study, we show through Monte Carlo simulations that, in most cases, it is sufficient that only between 1 % and 10 % of mixed water frog populations consist of R females to prevent or significantly reduce the fixation and accumulation of deleterious mutations.

Introduction

Ever since it has been postulated that sexual reproduction bears a two-fold reproductive disadvantage over asexual reproduction (Maynard Smith, 1971), evolutionary biologists have tried to identify benefits of sexual reproduction which might balance the cost of producing males. There are numerous hypotheses to explain why sexual reproduction dominates, despite the fact that all other things being equal, asexuals would have twice the reproductive rate of sexuals (reviewed by Kondrashov, 1993; West *et al.*, 1999). Several of these models see the main advantage of sexual reproduction in eliminating deleterious mutations. The importance of such elimination, and hence the selection pressure favouring sexual reproduction, may differ among taxa, depending on how mutations interact and the rates of deleterious mutations per generation (U) which, in turn, are a function of generation time (Keightley & Eyre-Walker, 2000).

However, most of these models assume a dichotomy of either sexual or asexual reproduction. They ignore that 'asexual' organisms may slip in a little sex once in a while and, hence, are capable of profiting from both reproductive modes without having to pay the inherent costs of producing large numbers of males. Typical examples for such mixed reproductive modes come from cyclical parthenogens, like aphids, cladocerans, rotifers and cynipid wasps, which alternate between several asexual and rare sexual generations (Rispe & Pierre, 1998; Atkinson *et al.*, 2003; Stelzer, 2005). Among small 'asexuals' with poorly investigated life cycles the phenomenon may be more common than generally perceived. It is not intuitively obvious, however, why mixed reproductive modes have not been documented more often for the usually well-studied large metazoans. In vertebrates a mix of sexual and asexual reproduction is largely absent, with the possible exception of a few

Correspondence: H.-U. Reyer, Institute of Zoology, University of Zurich, Winterthurerstr. 190, CH – 8057 Zurich, Switzerland.
Tel.: 41 1 6354980; fax: 41 1 6356821;
e-mail: ulireyer@zool.unizh.ch

Table 1 Mating combinations and the resulting offspring and their sex in mixed waterfrog populations.

	Males	
Females	*R. lessonae* (LL), $L_{m,f}$	*R. esculenta* (LR), R_f*
R. lessonae (LL), L_f	*R. lessonae* m, f	*R. esculenta* f
R. esculenta (LR), R_f*	*R. esculenta* m, f	*R. ridibunda* f
R. ridibunda (RR), R_f	*R. esculenta* m, f	*R. ridibunda* f

The letters in brackets show the genetic composition of the species. Single letters with indices indicate the type of genome that is present in the gametes, and the type of sex chromosome they can contain (m: male, f: female). Genomes of male and female *R. esculenta* (R_f*) are transmitted clonally, those of male and female *R. lessonae* and female *R. ridibunda* are recombined.

hybridogenetic species, namely teleost fishes of the genera *Poeciliopsis* (Vrijenhoek, 1989) and *Tropidophoxinellus* (Carmona *et al.*, 1997) and water frogs of the genus *Rana* which were the subject of this study.

The water frog system in central Europe consists mainly of the sexual parental species *Rana lessonae* (L) and the hybridogenetic species *R. esculenta* (E). *Rana esculenta* is not a 'true' species but a hybrid between *R. lessonae* and *R. ridibunda* (R) (cf. Table 1). In *R. esculenta*, recombination does not occur (Berger, 1977), because the one part of the hybrid's genome that has been inherited from *R. lessonae* is completely excluded from the germ line prior to meiosis (Graf & Müller, 1979; Uzzell *et al.*, 1980). The remaining R genome then undergoes a duplication followed by a normal meiosis (Tunner & Heppich-Tunner, 1991). Gametes of *R. esculenta* therefore only contain an unaltered haploid *R. ridibunda* set of chromosomes. Hybridity is restored by backcrossing with *R. lessonae*. This corresponds to a primary hybridization between the two parental species *R. lessonae* and *R. ridibunda*, but the latter is naturally absent from most parts of central Europe.

Due to the lack of recombination or genetic reshuffling between the sexual L (which is recombined within *R. lessonae*) and the clonal R genome, the hybrid's mode of reproduction is often called hemiclonal. As all gametes of the hybrid contain the same R genome, E × E matings result in diploid *R. ridibunda* offspring, but in most cases they are not viable (Semlitsch & Reyer, 1992). This is usually explained by high amounts of homozygote-recessive lethal factors that have accumulated in the clonal R genomes (e.g. Graf & Müller, 1979; Uzzell *et al.*, 1980) due to the operation of Muller's ratchet (Muller, 1964). Experimental results are, by and large, consistent with the operation of the ratchet. Vorburger (2001a,c) and Guex *et al.* (2002), for instance, found that *R. ridibunda* tadpoles with two different clonal R genomes and those with one clonal and one sexual R genome, on average, developed and survived better than those with two identical clonal R genomes.

Under this hemiclonal reproduction, the existence of males seems even more puzzling than in sexual species, because in hybridogens the demographic costs of males arising from reduced intrinsic rates of increase (compared with all female populations) are usually not compensated by genetic benefits arising from recombination. Sometimes, however, the usual intraspecific incompatibility in *R. esculenta* is broken up. Hotz *et al.* (1992) investigated the genetical properties of a *R. ridibunda* population living in sympatry with a mixed L–E population in a gravel pit in northern Switzerland. Based on two observations, they concluded that the *R. ridibunda* arose *in situ* from successful E × E matings, rather than from the several introductions of *R. ridibunda* into Switzerland. First, the mtDNA of the R females corresponds to the mtDNA of the sympatric *R. esculenta* and is significantly different from the mtDNA of introduced *R. ridibunda*. Secondly, the R population consisted of only females. This is because primary hybridizations occur mainly between L males and R females for size and behavioural reasons (Berger *et al.*, 1988), and sex is determined by an XX–XY system in water frogs, with males being heterogametic. As the paternal L genome is premeiotically eliminated, E male sperms can only contain the maternally inherited X–*ridibunda* chromosomes. Therefore, E males mating with L, R or E females produce all-female offspring. As the mtDNA of the R females corresponds to the mtDNA of the sympatric E population, matings of E males with introduced R females cannot be the source of the all-female R population studied by Hotz *et al.* (1992). More recently, Vorburger (2001b) confirmed the origin of all-female *R. ridibunda* from E × E matings for another Swiss population.

Although the lack of R males precludes the establishment of a self-sustaining R population, female *R. ridibunda* arising from E × E matings can play an important role for the genetic composition in mixed water frog populations: they are expected to show normal recombination (Hotz *et al.*, 1992) and their genome can be purged from deleterious mutations. If such females mate with L males, this gives rise to new hemiclonal E lineages (cf. Table 1) with novel gene combinations on their clonally transmitted R genome part. Based on the findings of Hotz *et al.* (1992), Schmidt (1993) compared *R. esculenta* populations that include some female *R. ridibunda* (hereafter called E–R_f populations) to cyclical parthenogens. He suggested that the genetic reshuffling described above has similar effects on the fitness of *R. esculenta* as the alternation of asexual and sexual reproduction in cyclical parthenogens. Milinski (1994) expressed doubts about the effectiveness of the rare successful E × E matings in reducing the deleterious mutation load in E–R_f populations, as natural *R. esculenta* populations often contain only one or a few hemiclonal R lineages. According to Milinski, recombination would then not be much more effective than selfing which cannot slow down Muller's ratchet (Heller & Maynard Smith, 1979; Lynch *et al.*, 1995).

Neither Schmidt nor Milinski could support their views with explicit data or results from models or simulations. In terms of data, some recent allozyme studies of natural L–E populations have documented coexistence of several hemiclonal R lineages within the same pond. Application of more variable microsatellite markers is likely to subdivide these hemiclones and, hence, increase their numbers even further (Semlitsch *et al.*, 1997; Vorburger, 2001b). The aim of this study was to simulate whether, under which conditions and to which extent the indirect reintroduction of recombination via sexual female *R. ridibunda* influences the fitness in *R. esculenta* populations.

However, recombination does not have advantages only. Recent investigations about sex differences in mutation rates show that in many sexual species, males have higher deleterious mutation rates than females (Hurst & Ellegren, 1998; McVean, 2000). These sex differences are supposed to be highest in externally fertilizing species where males have to produce vast numbers of sperms. Consequently, sperms should carry more new mutations than eggs. In the next generation, Mendelian segregation will result in new gametes which carry, on average, an intermediate number of the paternally (many) and the maternally (fewer) inherited deleterious mutations (plus any new mutations). Where males have a higher deleterious mutation rate than females, this 'cost of recombination' adds another disadvantage to the often cited two-fold costs of sex (Redfield, 1994).

In species that reproduce hybridogenetically such additional costs do not arise, as recombination, and hence averaging of male and female mutation rates through recombination, does not occur. In all-female species, which form the majority of hybridogens (Vrijenhoek, 1989), the clonally transferred genomes are only subjected to the lower female mutation rates. However, even where both sexes exist, as in hybridogenetic *R. esculenta*, the prevented recombination still could confer some advantage. As E males only sire female offspring, a clonally transferred R genome has spent, on average, more of its evolutionary history in females than in males and, as a consequence, the average deleterious mutation rate will be lower than in a comparable sexual species (Som, 2001).

In sexual species, the costs of recombination arising from higher male than female mutation rates thus have to be balanced against the benefits of preventing the fixation of deleterious mutations, which is the biggest threat for size-limited asexual populations. In the specific case of *R. esculenta*, successful $E \times E$ matings and the resulting recombining R females can contribute to maintaining the fitness, provided the number of fixed mutations throughout a small population is low compared with the average total number of deleterious mutations.

To our knowledge, no analytical solutions exist for the speed of Muller's ratchet in small hybridogenetic populations nor for the effects and the magnitude of drift. In addition to the two aforementioned stochastic processes, we incorporate the stochastic mutation transmission effects of Mendelian segregation. The development of an analytical solution that accounts for all three stochastic processes would most probably exceed the scope of a single study. We therefore make use of a Monte Carlo simulation to address the question if *R. esculenta* has found a way to circumvent Muller's ratchet without having to pay the two-fold cost of sex.

Simulations

In natural diploid $E–R_f$ populations, the observed sex ratio is often biased towards females (e.g. Berger, 1977; Holenweg Peter *et al.*, 2002), because E males sire only daughters (see Introduction). L males, on the other hand, produce offspring with an even sex ratio. Consequently, the sex ratio in $E–R_f$ populations is determined by the relative success of E males siring offspring with L or R females vs. the success of L males siring offspring with E and R females (Table 1). For our simulations, we assume that E males and E and R females have the same per capita number of offspring. This results in a stable E male to E + R female sex ratio of 1 : 2, neglecting the minor effect of the rare $E \times E$ and $R_f \times E_m$ matings on the sex ratio.

Starting with a mutation-free population, we followed the accumulation and fixation of mutations for 8000 generations. We chose this number of generations to cover the timespan between the last ice age and today. Severe climatic conditions during that period must have prevented the existence of frogs in central and northern Europe and confined them to areas in southern and south-eastern Europe. The present distribution of water frogs in central Europe is thus the result of recolonization from those refuge areas, and the history of the hemiclonal E lineages may go back to the time when recolonization began.

Our hypothetical $E–R_f$ population lives in sympatry with an infinitely large *R. lessonae* population with a 1 : 1 sex ratio, occupying a subniche with limited carrying capacity in the *R. lessonae* distribution area. We chose an unlimited L population size with the purpose to separate small mixed L–E population dynamics artefacts from the problem of accumulation of deleterious mutations in hemiclonal lineages. The population dynamics of small mixed L–E populations are investigated in Som *et al.* (2000). The L population is in mutation-selection balance which means that the frequencies of classes of individuals with 0, …, n deleterious mutations remain constant over time. We accounted for differences in male and female mutation rates, by varying the male : female mutation rate ratio (α) between 1 and 10 (see 'Testing conditions'). The E and R females show the same genomic deleterious mutation rate as L females and E males the same as L males.

We simulated reproduction in E–R_f populations by randomly combining eggs and sperms from a large pool of gametes, thus following an extended Wright–Fisher approach (Wright, 1931). Although females show a preference for L males in two-fold choice experiments (Abt & Reyer, 1993; Roesli & Reyer, 2000; Engeler & Reyer, 2001), their preference is overrun by competition among indiscriminately mating males under more realistic conditions (Bergen *et al.*, 1997). Hence, random mating in nature seemed a reasonable assumption. Generations in our model do not overlap and population size remains constant from generation to generation. As selection is assumed to act on gamete production (i.e. fertility), an individual's contribution to this pool is proportional to its fitness relative to the other individuals.

R females originate from E males mating with either E or R females ($E_f \times E_m$ or $R_f \times E_m$, see Table 1). In the latter case, the female *R. ridibunda* show normal Mendelian segregation and each mutation has a 50 % chance (100 % in the case of homozygosity for the specific mutation) of being present in eggs. All the mutations on the E father's clonally transmitted part of its genome are present in the sperm. In the homospecific mating, both parents transfer all their mutations residing on the clonally transmitted R genome parts to their offspring.

Under the demographic mechanisms described above and an assumed sex ratio of 1 : 2 males to females, half of the E females originate from E or R females mating with male *R. lessonae* ($E_f \times L_m$ or $R_f \times L_m$, see Table 1). As no crossing over occurs between the L and R genomes in the resulting offspring (Schultz, 1969), it is not necessary to follow the fate of individual mutations on the father's L sperm. Only the total number of mutations has to be considered, as we assume that both the mutations on the L part and the mutations on the R part of a *R. esculenta*'s genome affect its fitness. If an R female mates with an L male, this generates a new hemiclonal E lineage. Again, each of the mother's mutation has a 50 % chance of being present in the egg and thus later on in the new hemiclonal lineage. $E_f \times L_m$ matings do not generate new lineages but simply perpetuate the hemiclone of the mother combined with a new L mutational background. The other half of the E females arises from matings between L females and E males ($L_f \times E_m$, see Table 1). In this case, the paternal hemiclonal R lineage is passed on to the offspring and there combined with an L maternal part.

E males originate from E or R females mating with L males (see Table 1). $R_f \times L_m$ matings produce new hemiclonal lineages whereas $E_f \times L_m$ matings perpetuate the maternal hemiclonal R lineage.

L males and females originate from intraspecific *R. lessonae*, but are not considered in the simulation as we assume an infinitely large L population (see above).

After the new E–R_f is completed, all individuals undergo mutation accumulation. In our model, we assume a total of 1000 loci susceptible to deleterious mutations per genome. We kept track of individual mutations throughout the generations so that we were able to test for drift effects or fixation events. The probability of acquiring new deleterious mutations follows a Poisson distribution with the mean of U (Kimura & Maruyama, 1966), where U is the average number of new deleterious mutations an individual acquires during its lifespan. Note that U can be different for males and females and that mutations can be recurrent. We assume here that the effect deleterious mutations have on fitness is independent of the locus where they occur. Homozygous mutations at one locus have the same effect on fitness as two heterozygous mutations on different loci. New mutations in an E individual are equally likely to occur on the clonally transmitted R part as on the sexual part of their genome, but only the ones on the R part can be passed on to the new generation (cf. Table 1).

Testing conditions

As stochastic effects like drift and the fixation of mutations are population size dependent, we tested two different E–R_f population sizes in our model: a large population with 2700 individuals (1800 females, 900 males) and a small population with 270 individuals (180 females, 90 males). Three different model modes of mutation interactions (Redfield, 1994; Som, 2001) with variable degrees of epistasis were used (Fig. 1): independent interaction (no epistasis), and two levels of synergistic interaction, quadratic (moderate epistasis)

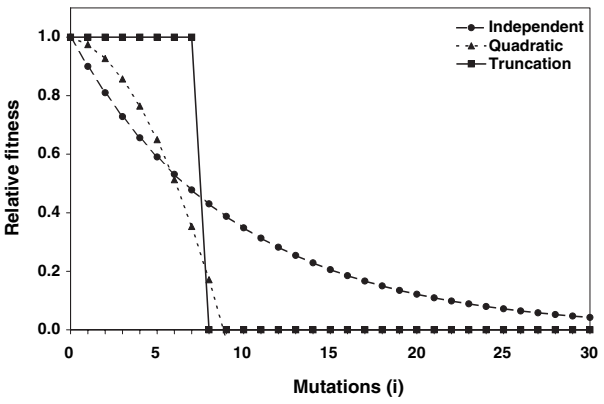

Fig. 1 Three representative types of selection against mutations with variable degrees of synergistic epistasis (after Redfield, 1994). The vertical axis shows the relative fitness of an individual with i deleterious mutations compared with an individual with no deleterious mutations. Independent selection: relative fitness rf = 0.9^i. Quadratic selection: rf = $1 - 0.014i - 0.0112i^2$. Truncation selection: rf = 1 if $i \leq 6$, else rf = 0. The independent and truncation selection were chosen to represent no and extreme epistasis, respectively, with selection intensities equivalent to that of the biologically realistic moderate epistasis represented by the quadratic selection (cf. Crow, 1970).

and truncation (high epistasis). Empirical support for the two levels of synergistic interaction is scarce and controversial (West *et al.*, 1999; Agrawal & Chasnov, 2001; Burch *et al.*, 2003) and even the methods for experimental testing of epistasis are debated (West *et al.*, 1998). Yet, the existence of epistasis has been firmly established through population genetic studies (Fenster *et al.*, 1997), there is some theoretical support for synergistic epistasis (Szathmary, 1993; Peck & Waxman, 2000) and its effect, namely the elimination of deleterious mutations 'in bunches', has been suggested as an explanation why the observed levels of mutation rates do not inevitably lead to extinction (Crow, 1999). Therefore, we believe that for the purpose of this model our assumptions about mutation interactions are justified.

We held the female mutation rate U constant at 0.3 deleterious mutations per individual and generation. For the male mutation rate, we multiplied the female mutation rate by the factors 1, 2, 6 and 10 which are the most often mentioned values for α (the male to female mutation rate ratio) from studies in insects, rodents, primates and humans (reviewed by Hurst & Ellegren, 1998). In our model populations, we varied the percentage of female *R. ridibunda* between 0 % (no successful E × E matings), 0.1 %, 1 % and 10 % of the whole population (males + females). We tested all possible combinations between population size, type of mutation interaction, α and percentage of R females in the population. After 8000 generations, we noted the relative fitnesses of the male and female subpopulations compared with mutation-free populations and recorded the average number of mutations and the number of fixed mutations on the clonally transmitted R genome parts in the population. As our model is probabilistic, we repeated each parameter combination 10 times and calculated the average and the standard deviation from these 10 runs.

Results

Independent interaction

Small populations

As there is no cumulative effect of mutations under independent mutation interaction, the average number of mutations in an individual is usually much higher than under the other selection regimes. In model populations with a male-to-female mutation rate ratio α of 10, the simulations stopped before the end of the normal simulation time of 8000 generations because of zero population fitness. At lower α values, population fitness depended on the proportion of recombining females of *R. ridibunda* present in E × R_f populations. With no or very few female *R. ridibunda* (0 % and 0.1 % R_f), mutations accumulate to such an extent (Fig. 2a) that the average fitness of the model populations is reduced to a tenth of their maximum fitness (Fig. 2b). With 1 % of sexual R females, average population fitness rises to 23 % for the case where male and female mutation rates are equal ($\alpha = 1$). However, the average number of fixed mutations almost equals the total average number of mutations on the R genome part (Fig. 2a). This indicates, that under these conditions, Muller's ratchet is still operating but at a reduced speed.

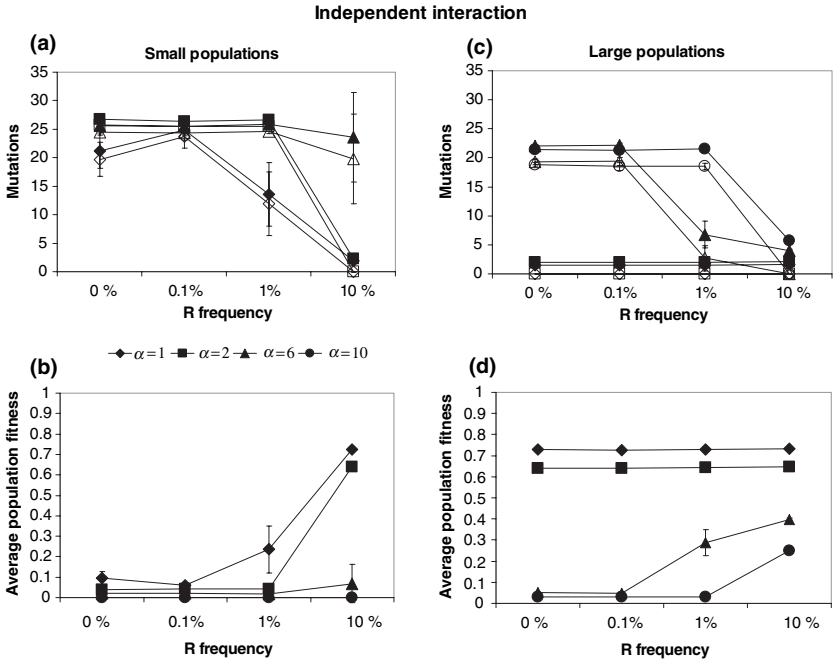

Fig. 2 Number of mutations on the clonally transmitted R genome (top) and the average population fitness (bottom) for small (left) and large (right) populations under independent selection in relation to the composition of a mixed E–R_f population and the male-to-female mutation rate α. Closed symbols in the upper graph represent the total number of mutations on the R genome, whereas open symbols represents how many of these are fixed in the whole population. Horizontal axes indicate the percentage of the total E–R_f population (males + females) that consists of female *R. ridibunda*. The vertical axis on the lower graph shows the relative fitness of the E–R_f population compared with the fitness of an ideal mutation-free population. Symbols represent the different levels of α indicated in the graph. Under conditions where the average population fitness is zero (lower graphs), the respective symbol cannot – by definition – be shown in the upper graphs.

At higher α levels, the benefits of recombination are not sufficient to compensate for the higher average mutation rate caused by the presence of males. Hence, the average population fitness remains very low, unless the R_f proportion is 10 % (Fig. 2b). At 10 % R females, the number of total and fixed mutations drops (Fig. 2a) and the average population fitness increases markedly for the lower two values of α. The fitness of the small model populations approximately equals the fitness ($W_{eq} = e^{-U} = 0.74$ for $U = 0.3$) of infinite asexual populations and sexual populations with $\alpha = 1$ in mutation-selection balance (Kimura & Maruyama, 1966). At the same time, no mutations were fixed for $\alpha = 1.2$ and R_f frequency of 10 %. At higher male-to-female mutation rate ratios, a 10 % fraction capable of recombination of the population was not high enough to prevent the accumulation of mutations.

Large populations

For $\alpha = 1$ and $\alpha = 2$, mutation accumulation in a population size of 2700 is so low (Fig. 2c) that it does not pose a major threat to population fitness (Fig. 2d). All model populations with these two α levels maintained a high fitness level, regardless of the amount of recombination which is determined by the proportion of R females. At higher male-to-female mutation rate ratios, mutation accumulation remains a critical aspect for population fitness unless there are at least 1 % female *R. ridibunda* (Fig. 2c). Model E–R_f populations with an α of 6 reach approximately the same fitness as an infinite sexual population with the same α and U [$W_{eq} = e^{-U(1+\alpha)/2} = 0.35$ for $U = 0.3$ and $\alpha = 6$]. Populations with $\alpha = 10$ are not viable under independent mutation interaction, unless mixed E–R_f populations consist of at least 10 % R females (Fig. 2d). At that level, however, the fitness of such E–R_f populations exceeds the theoretical fitness of comparable infinite sexual populations ($W_{eq} = 0.19$) by a factor of 1.3. At 10 % R females in the mixed population, no fixation of mutations occurred at any level of α (Fig. 2c).

Quadratic interaction

Small populations

If mutations interact synergistically, the overall number of mutations remains generally much smaller than under independent interaction. Consequently, the scale of the *y*-axis in the upper graph of Figs 3 and 4 differs from the one in Fig. 2. Overall, the number of fixed mutations decreases with an increasing male-to-female mutation rate α (Fig. 3a). This is because of an increasing shielding effect against the accumulation of deleterious mutations on the clonally transmitted genome part intrinsic to hybridogenetically reproducing organisms (see Introduction). At all four α levels, mutation rates and average population fitness are relatively unaffected by R female proportions as long as these are between 0 % and 1 % (Fig. 3a,b). At $R_f = 10$ % the situation is different. Here, the average number of mutations is reduced, fixation of mutations does not occur at all and average population fitness is higher for all levels of α. It is only under these conditions that small populations with an α of 10 become

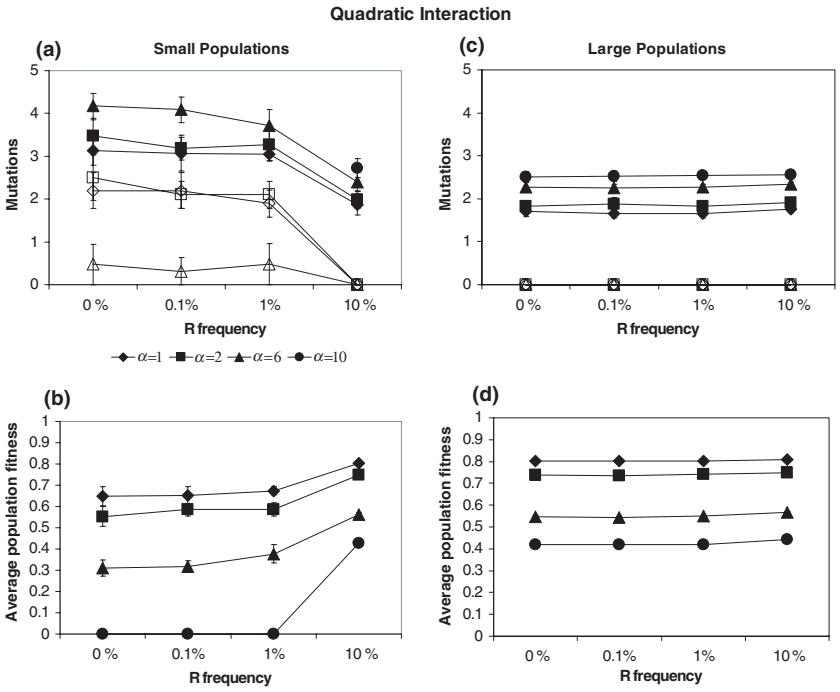

Fig. 3 Number of mutations and average population fitness for small and large E–R_f populations under quadratic selection. For details see legend to Fig. 2.

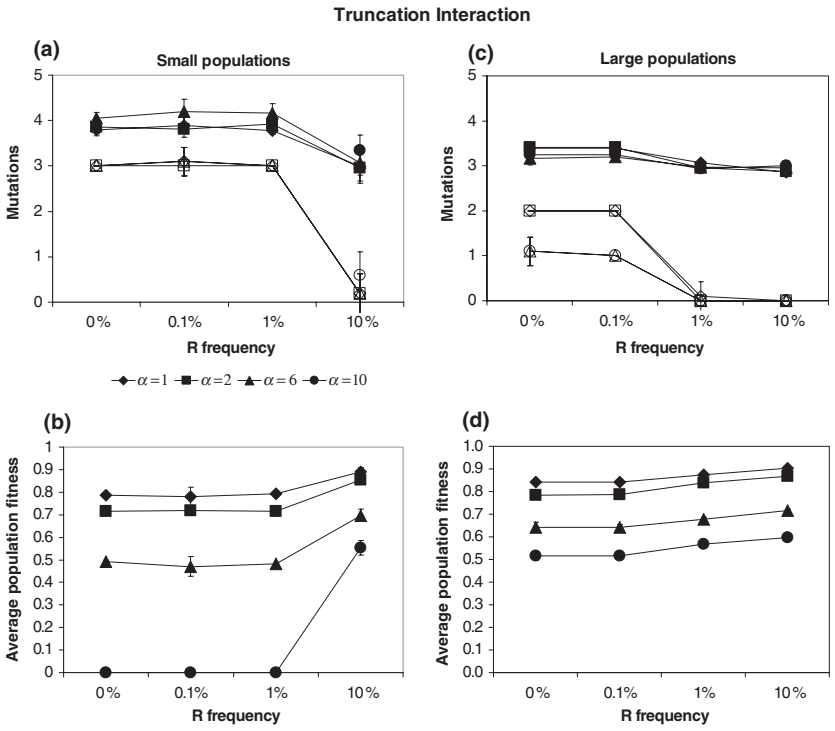

Fig. 4 Number of mutations and average population fitness for small and large E–R_f populations under truncation selection. For details see legend to Fig. 2.

viable; but then their fitness exceeds the fitness of populations with an α of 6 at lower R female frequencies. The fitness of E–R_f populations with 10 % R females corresponds to the fitness of infinite sexual populations with the same values of α (Redfield, 1994).

Large populations

In large E × R_f populations, selection against new mutations is strong enough to prevent substantial accumulation of deleterious mutations under all α values, and fixation does not occur at all (Fig. 3c). The presence or absence of female *R. ridibunda* in the mixed population has no influence on the average number of mutations (Fig. 3c) or on the average population fitness (Fig. 3d). Average population fitnesses correspond to the fitnesses of sexual populations with the same α.

Truncation selection

Small populations

Overall, the pattern under truncation selection closely resembled the one under quadratic interaction for both, small and large E–R_f populations. In small populations, where mutations show truncation interaction, R frequencies below 10 % have little influence on population fitness for any of the four α values (Fig. 4b). At 10 % R females, however, the average number of deleterious mutations drops slightly for all α values (Fig. 4a) which increases the fitness for populations with α levels of 1, 2 and 6 by 13 %, 19 % and 45 % respectively. Populations with an α of 10 are only viable if the population consists of at least 10 % female *R. ridibunda*. The most noticeable change after altering R female proportions from 1 % to 10 %, is the almost complete disappearance of fixation of mutations for all values of α.

Large populations

Contrary to the small populations, large populations with a male-to-female mutation rate ratio of 10 are viable under all R frequencies in large populations, and so are populations with α values of 0 %, 0.1 % and 1 % (Fig. 4d). For all four α values, the average population fitness increases slightly with higher R frequencies. Similar to quadratic interaction, the number of fixed mutations on the R genome part is negatively correlated with α at the lower two R frequencies. At R frequencies of 1 % and higher, fixation of mutations is successfully prevented. At R frequencies of 10 %, average population fitnesses lay well within the range of comparable sexual species (Redfield, 1994; Som, 2001).

Discussion

With this study, we have been able to show that the reintroduction of recombination into an otherwise hybridogenetic or hemiclonal reproductive system can have a clearly beneficial effect on the fecundity of a small population. Using the example of mixed E–R_f water frog populations, we could show that, in most cases, it is sufficient that only between 1 % and 10 % of the

population consists of R females capable of recombination. This low percentage of sexual matings necessary to maintain fitness in a hybridogenetic population agrees well with the results from studies of true cyclical parthenogens which have shown that a few generations of sexual reproduction among many generations of asexual reproduction are sufficient to maintain fitness and viability in small populations (Charlesworth *et al.*, 1993; Green & Noakes, 1995). If deleterious mutations interactions do not show epistasis (independent interaction, Fig. 2), the presence of recombining R females is even a prerequisite for the longtime persistence of small E–R_f populations.

Our results also contradict Milinski's (1994) argument that recombination would not prevent Muller's ratchet from operating because of the selfing nature of E × E matings. In several cases, such as quadratic and truncation selection in small populations and truncation interaction in large populations (Figs 3 and 4), recombination prevented or significantly reduced the number of fixed mutations. This reduction in the number of fixed mutations was even stronger than the reduction of the overall number of mutations on the R genome as can be deducted from the steepness of the trajectories from 1 % to 10 % (Fig. 4a,c). Notably, recombination in R females has a positive effect on the fitness and the number of fixed mutations, even if E–R_f populations are isolated. We did not include the effect of immigration of different E hemiclones into the model populations, but this would increase the recombination rate even further (Vorburger, 2001b) and provide additional benefits.

Large asexual populations where females have the same genomic deleterious mutation rate as females in a sexual population can have a lower average mutation load than sexual populations if males show a higher deleterious mutation rate than females (Redfield, 1994). The same applies for large unisexual hybridogenetic populations (Som, 2001). In the E–R_f system, this advantage of unisexuality in terms of the average population fitness is no longer perceivable in the large model populations, despite the reduced frequency of recombination and the skewed sex ratio with a surplus of females. Instead, the fitness of E–R_f populations with a fraction of 10 % R females is comparable with the fitness of large sexual populations with the same sex-specific deleterious mutation rates (Redfield, 1994). But as half of a *R. esculenta*'s genome consists of a sexual L part, the mutation accumulation dynamics of the clonally propagated R part are not responsible alone for the overall fitness of an E population. Yet, the reduction in frequency of recombination compared with sexual populations and the skewed sex ratio still have a beneficial effect. In large E–R_f populations under quadratic or truncation interaction, the amount of deleterious mutations on the clonally transmitted R genome stays virtually the same, regardless of the male-to-female mutation rate α (Figs 3c and 4c).

A small amount of recombination is necessary to slow down Muller's ratchet or to prevent the fixation of deleterious mutations in small populations. Interestingly, the small E–R_f populations with 10 % R females can maintain the same level of fitness as sexual populations. Thus, in small populations, the investment of producing E males, which in turn can generate R females, pays off. This is especially valid if one considers that E males produce all female offspring, which balances the costs of producing a son by getting two granddaughters in return. Furthermore, in sexual populations, the first-generation offspring of a female carries half of the mother's alleles and the second-generation offspring (the combined offspring of sons and daughters) only one-fourth. In E–R_f populations on the other hand, the R genome part of an E mother mating with an L male is propagated unaltered by sons and daughters. Because in E–R_f populations, living in sympatry with sexual populations, interspecific matings are much more common than successful intraspecific E matings, chances are high that the combined second-generation offspring still carry the same R alleles as their grandmother. In this case, the costs of producing sons are uncoupled from the costs of recombination (i.e. the disruption of maternal genomes).

The plausibility of the above statements hinges, of course, on how realistic our mutation and selection models are. Assuming a diploid mutation rate $U = 0.3$ with homozygous effects $s = 0.1$ will – without selection – result in a fitness decline through mutation accumulation of 1.5 % (=$0.1 \times 0.3/2$). This is a fairly strong mutation impact (cf. Garcia-Dorado *et al.*, 1999) and, hence, may overestimate the selective benefits from purging through recombination. The same is true for our assumption that all mutations have additive and constant homozygous effects of $s = 0.1$. If some mutations were recessive and/or their effects were variable around a mean of $s = 0.1$, purging would be less effective. However, although higher than most published values, our assumptions are still within the range found and used in other studies. Estimates for mutation rates (U) differ by several orders of magnitude (Baer *et al.*, 2005). For *Drosophila melanogaster* they range from 0.01 to 1.0 (cited from Gong *et al.*, 2005), and for hominids Eyre-Walker & Keightley (1999) report average U values as high as 1.2–1.6. Homozygous effects of $s = 0.1$ are often used in models like ours (e.g. Redfield, 1994; Morgan, 2002), and Shabalina *et al.* (1997) found fitness declines in *Drosophila* of up to 2 % per generation. Compared with other laboratory studies that found fitness declines between 0.1 % and 1.0 % (Chavarrías *et al.*, 2001), 2 % is, indeed, an outlier. It is known, however, that deleterious mutations can have larger effects in harsh (i.e. natural) environments than under benign (i.e. laboratory) conditions (e.g. Shabalina *et al.*, 1997; Szafraniec *et al.*, 2001) and that inbreeding depressions can be stronger under stressful circumstances (reviewed by Keller & Waller, 2002). Hence, in natural frog

populations homozygous effects of $s = 0.1$ and a 1.5 % fitness decline through mutation accumulation seem to be a realistic assumption. Moreover, even a substantial reduction in parameter values is unlikely to change our result. Chasnov (2000) and Agrawal & Chasnov (2001) have shown that the fitness of sexuals (W_{sex}) relative to asexuals (W_{asex}) is sensitive to the levels of the dominance coefficient (h) and the selection coefficient (s), but the effects of h and s on W_{sex}/W_{asex} decrease with increasing population subdivision, measured by Wright's inbreeding coefficient (f). Even with moderate population genetic structure (e.g. $f = 0.1$), results for $s = 0.1$ and $s = 0.01$ do not differ. And with the marked population subdivision often found in philopatric amphibians ($f = 0.25$–0.85; Hitchings & Beebee, 1997; Formas & Brieva, 2000) the effects of h-values ranging from 0.4 to 0.01 converge, too (see Fig. 2 in Agrawal & Chasnov, 2001).

These data and considerations, plus the fact that realistic values for U and s are lacking for amphibians (and most other animals) and that we have no reasonable estimates of dominance, strength of mutation effects and modes of gene interaction, prompted us to choose parameter values, degrees of synergistic epistasis and selection types (independent, quadratic, truncation) that allow comparison of predictions between our and other models (e.g. Redfield, 1994; Whitlock & Bourguet, 2000). Moreover, conditions that favour purging make our conclusions only stronger. With a strong effect of recombination, one would expect that small sexual populations do much better than hybridogenetical populations, but we find little difference in fitness between sexual populations and hybridogenetic populations with occasional recombination. With a reduced recombination effect, this difference should even become smaller.

In addition to the assumed genetic conditions, our demographic assumptions that generations are nonoverlapping and population size is constant through time must have influenced the results and conclusions from this study. In real, substantially fluctuating populations, an increase in animal numbers can enhance the fixation probability for beneficial mutations (Otto & Whitlock, 1997), whereas a decrease in numbers can make fixation of deleterious mutations more likely (mutational meltdown). The negative effects of such a genetic bottleneck can be mitigated when generations are overlapping (Kuo & Janzen, 2003). However, with overlapping generations, increased mutational load on the gametes of older E males may counterbalance the mitigating effect. And given that even a 10-fold size difference between large and small populations does not qualitatively alter the conclusion that 10 % R females are sufficient to maintain population viability, we doubt that realistic fluctuations in population size would markedly change results and conclusions from the model.

Having shown that in theory, recombining R females that arose from E × E matings can play an important role in E–R_f populations living in sympatry with *R. lessonae*, the question remains how frequent such mixed E–R_f populations are in nature. As field surveys of amphibian distribution are often made by recording calls of males (Heyer *et al.*, 1994), the presence of female *R. ridibunda* in *R. esculenta* populations may sometimes have escaped detection. However, apart from the population described by Hotz *et al.* (1992), there is evidence for at least one other case in Switzerland. Vorburger (2001b) investigated a population in Elliker Auen near Ellikon, Kanton of Zurich, Switzerland. He not only showed experimentally that E × E matings can produce viable R females but also found sexual all-female *R. ridibunda* metamorphs in the field whose genotypes could be clearly assigned to the hemiclonal lineages present in that population. However, during the 3 years of the investigation (1998–2000), *R. ridibunda* metamorphs were only found in 1999, juveniles only in 2000 and adults in none of the three years. As 1999 was exceptionally favourable for tadpole development due to a flooding of the study area early in the season, *R. ridibunda* larvae may successfully complete metamorphosis only when competition with *R. lessonae* and *R. esculenta* is reduced. This, plus the fact that *R. ridibunda* seemed to survive the first winter in the field less well than *R. lessonae* and *R. esculenta*, led Vorburger (2001b) to 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' (see also Milinski, 1994). Only when and where ecological conditions are benign for *R. ridibunda* for an extended period, as probably in the population described by Hotz *et al.* (1992), some R females may eventually reach sexual maturity. As the frequency of recombination needed to maintain fecundity in E–R_f populations is relatively low, a few R cohorts reaching maturity may be able to guarantee sufficient genetic diversity.

The reasons for the lower survival of *R. ridibunda* originating from E × E matings are presently unknown. The consequences, however, are that, in spite of the 'selfish' behaviour of the R genome, female *R. ridibunda* will not spread and, in the absence of conspecific males, finally lead the population to extinction. If, however, ecological conditions become very favourable for *R. ridibunda* and if R males are present in the population (e.g. introduced by humans), the hybridogenetic mode of reproduction will lead to the total replacement of *R. lessonae* and *R. esculenta* through *R. ridibunda* (Vorburger & Reyer, 2003).

But are the effects described above sufficient to convert *R. esculenta* from 'no hopers to hopeful monsters' (Vrijenhoek, 1989)? We are not at a stage yet where this can be answered with sufficient confidence as some important questions could not be addressed in this study. What causes the often observed reproductive incompatibility in E × E matings? Our simulation suggests that this phenomenon cannot be explained with the mutational load alone. How does this incompatibility change

with the number of hemiclones present? Do successful E × E matings and the recombining R females trigger a positive feedback loop fed through the elimination of deleterious mutations, which then produces healthier hemiclones, which in turn increase the chances of successful E × E matings, and so on? In spite of these and other remaining questions, we think that if there are candidates that can escape the postulated evolutionary dead end of asexuality among higher organisms (Maynard Smith, 1978), *R. esculenta* certainly is among them.

Acknowledgments

We are grateful to R. Altwegg, T. Garner, P. Pearman, B. Schmidt, J. Van Buskirk, C. Vorburger and all other people from our laboratory who, directly or indirectly, contributed to this study and the resulting manuscript through interesting ideas and fruitful discussions. Valuable comments from two anonymous reviewers improved the manuscript considerably. The study was supported by the Swiss National Fund (grant no. 31-40688.94 to H.-U.R.).

References

Abt, G. & 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.

Agrawal, A.F. & Chasnov, J.R. 2001. Recessive mutations and the maintenance of sex in structured populations. *Genetics* **158**: 913–917.

Atkinson, R.J., Brown, G.S. & Stone, G.N. 2003. Skewed sex ratios and multiple founding in galls of the oak apple gall wasp *Biorhiza pallida*. *Ecol. Entomol.* **28**: 14–24.

Baer, C.F., Shaw, F., Steding, C., Baumgartner, M., Hawkins, A., Houppert, A., Mason, N., Reed, M., Sinnonelic, K., Woodard, W. & Lynch, M. 2005. Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 5785–5790.

Bergen, K., Semlitsch, R.D. & Reyer, H.-U. 1997. Hybrid female matings are directly related to the availability of *Rana lessonae* and *Rana esculenta* males in experimental populations. *Copeia* **1997**: 275–283.

Berger, L. 1977. Systematics and hybridization in the *Rana esculenta* complex. In: *The Reproductive Biology of Amphibians* (D. H. Taylor & S. I. Guttman, eds), pp. 376–388. Plenum Press, New York.

Berger, L., Uzzel, T. & Hotz, H. 1988. Sex determination and sex ratios in western Palearctic water frogs: XX and XY hybrids in the Pannonian Basin?. *Proc. Acad. Nat. Sci. Philadelphia* **140**: 220–239.

Burch, C.L., Turner, P.E. & Hanley, K.A. 2003. Patterns of epistasis in RNA viruses: a review of the evidence from vaccine design. *J. Evol. Biol.* **16**: 1223–1235.

Carmona, J.A., Sanjur, O.I., Doadrio, I., Machordom, A. & Vrijenhoek, R.C. 1997. Hybridogenetic reproduction and maternal ancestry of polyploid Iberian fish: the *Trophidophoxinellus alburnoides* complex. *Genetics* **146**: 983–993.

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

Chasnov, J.R. 2000. Mutation-selection balance, dominance and the maintenance of sex. *Genetics* **156**: 1419–1425.

Chavarrías, D., Lopez-Fanjul, C. & Garcia Dorado, A. 2001. The rate of mutation and the homozygous and heterozygous mutational effects for competitive viability: a long-term experiment with *Drosophila melanogaster*. *Genetics* **158**: 681–693.

Crow, J.F. 1970. Genetic loads and the cost of natural selection. In: *Mathematical Topics in Population Genetics* (K. Kojima, ed), pp. 128–177. Springer-Verlag, Berlin.

Crow, J.F. 1999. The odds of loosing at genetic roulette. *Nature* **397**: 293–294.

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

Eyre-Walker, A. & Keightley, P.D. 1999. High genomic deleterious mutation rates in hominids. *Nature* **297**: 344–347.

Fenster, C.B., Galloway, L.F. & Chao, L. 1997. Epistasis and its consequences for the evolution of natural populations. *Trends Ecol. Evol.* **12**: 282–287.

Formas, J.R. & Brieva, L.M. 2000. Population genetics of the Chilean frog *Batrachyla leptopus* (Leptodactylidae). *Genet. Mol. Biol.* **23**: 43–48.

Garcia-Dorado, A., Lopez-Fanjul, C. & Caballero, A. 1999. Properties of spontaneous mutations affecting quantitative traits. *Genet. Res.* **74**: 341–350.

Gong, Y., Woodruff, R.C. & Thompson, J.N. 2005. Deleterious genomic mutation rate for viability in *Drosophila melanogaster* using concomitant sibling controls. *Biol. Lett.* **1**: 492–495.

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

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

Guex, G.-D., Hotz, H. & Semlitsch, R.D. 2002. Deleterious alleles and differential viability in progeny of natural hemiclonal frogs. *Evolution* **56**: 1036–1044.

Heller, R. & Maynard Smith, J. 1979. Does Muller's ratchet work with selfing? *Genet. Res.* **32**: 289–293.

Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.-A.C. & Foster, M.S. 1994. *Measuring and Monitoring Biological Diversity – Standard Methods for Amphibians*. Smithsonian Institution Press, Washington and London.

Hitchings, S.P. & Beebee, T.J.C. 1997. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* **79**: 117–127.

Holenweg Peter, A.-K., Reyer, H.-U. & Abt Tietje, G. 2002. Ecological factors affecting genotype and sex ratios in mixed populations of hybridogenetic water frogs. *Ecoscience* **9**: 1–11.

Hotz, H., Beerli, P. & Spolsky, C. 1992. Mitochondrial DNA reveals formation of nonhybrid frogs by natural matings between hemiclonal hybrids. *Mol. Biol. Evol.* **9**: 610–620.

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

Keightley, P.D. & Eyre-Walker, A. 2000. Deleterious mutations and the evolution of sex. *Science* **290**: 331–333.

Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**: 230–241.

Kimura, M. & Maruyama, T. 1966. The mutational load with epistatic gene interactions in fitness. *Genetics* **54**: 1337–1351.

Kondrashov, A.S. 1993. Classification of hypotheses on the advantage of amphimixis. *J. Hered.* **84**: 372–387.

Kuo, C.H. & Janzen, F.J. 2003. A bottleneck simulation program for long-lived species with overlapping generations. *Mol. Ecol. Notes* **3**: 669–673.

Lynch, M., Conery, J. & Burger, R. 1995. Mutational meltdowns in sexual populations. *Evolution* **49**: 1067–1080.

Maynard Smith, J. 1971. What use is sex?. *J. Theor. Biol.* **30**: 319–335.

Maynard Smith, J. 1978. *The Evolution of Sex*. Cambridge University Press, Cambridge, UK.

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

Milinski, M. 1994. Hybridogenetic frogs on an evolutionary dead end road. *Trends Ecol. Evol.* **9**: 62.

Morgan, M.T. 2002. Genome-wide deleterious mutation favors dispersal and species integrity. *Heredity* **89**: 253–257.

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

Otto, S.P. & Whitlock, M.C. 1997. The probability of fixation in populations of changing size. *Genetics* **146**: 723–733.

Peck, J.R. & Waxman, D. 2000. Mutation and sex in a competitive world. *Nature* **406**: 399–404.

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

Rispe, C. & Pierre, J.-S. 1998. Coexistence between cyclical parthenogens, obligate parthenogens and intermediates in a fluctuating environment. *J. Theor. Biol.* **195**: 97–110.

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

Schmidt, B.R. 1993. Are hybridogenetic frogs cyclical parthenogens? *Trends Ecol. Evol.* **8**: 271–273.

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

Semlitsch, R.D. & Reyer, H.-U. 1992. Performance of tadpoles from the hybridogenetic *Rana esculenta* complex: Interactions with pond drying and interspecific competition. *Evolution* **46**: 665–676.

Semlitsch, R.D., Hotz, H. & Guex, G.-D. 1997. Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the Frozen Niche Variation model. *Evolution* **51**: 1249–1261.

Shabalina, S.A., Yampolsky, L.Y. & Kondrashov, A.S. 1997. Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 13034–13039.

Som, C. 2001. Evolutionary Perspectives of the Hybridogenetic Waterfrog *Rana esculenta*. PhD Thesis, University of Zürich, Zürich.

Som, C., Anholt, B.R. & 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.

Stelzer, C.P. 2005. Evolution of rotifer life histories. *Hydrobiologia* **546**: 335–346.

Szafraniec, K., Borts, R.H. & Korona, R. 2001. Environmental stress and mutational load in diploid strains of the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **98**: 1107–1112.

Szathmary, E. 1993. Do deleterious mutations act synergistically? Metabolic control theory provides a partial answer. *Genetics* **133**: 127–132.

Tunner, H.G. & Heppich-Tunner, S. 1991. Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. *Naturwissenschaften* **78**: 32–34.

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

Vorburger, C. 2001a. Heterozygous fitness effects of clonally transmitted genomes in waterfrogs. *J. Evol. Biol.* **14**: 602–610.

Vorburger, C. 2001b. Non-hybrid offspring from matings between hemiclonal hybrid waterfrogs suggest occasional recombination between clonal genomes. *Ecol. Lett.* **4**: 628–636.

Vorburger, C. 2001c. Fixation of deleterious mutations in clonal lineages: evidence from hybridogenetic frogs. *Evolution* **55**: 2319–2332.

Vorburger, C. & Reyer, H.-U. 2003. A genetic mechanism of species replacement in European waterfrogs? *Conserv. Genet.* **4**: 141–155.

Vrijenhoek, R.C. 1989. Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. In: *Evolution and Ecology of Unisexual Vertebrates* (R. M. Dawley & J. P. Bogart, eds), pp. 24–31. Bulletin 466, The New York State Museum, Albany, NY.

West, S.A., Peters, A.D. & Barton, N.H. 1998. Testing epistasis between deleterious mutations. *Genetics* **149**: 435–444.

West, S.A., Lively, C.M. & Read, A.F. 1999. A pluralist approach to sex and recombination. *J. Evol. Biol.* **12**: 1003–1012.

Whitlock, M.C. & Bourguet, D. 2000. Factors affecting the genetic load in *Drosophila*: synergistic epistasis and correlations among fitness components. *Evolution* **54**: 1654–1660.

Wright, S. 1931. Evolution in Mendelian populations. *Genetics* **16**: 97–159.

Received 11 April 2006; revised 17 August 2006; accepted 22 August 2006