Choosy females and indiscriminate males: mate choice in mixed populations of sexual and hybridogenetic water frogs (*Rana lessonae, Rana esculenta*)

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For several decades, behavioral ecologists have studied the effects of the environment on the behavior of individuals; but only fairly recently they have started to ask the reverse question: how do the behavioral strategies of individuals affect the composition and dynamics of populations and communities? Although intuitively obvious, this feedback from individual to higher levels is difficult to demonstrate, except in systems with exceptionally fast and marked responses of the populations to the behavior of its members. Such a system exists in sperm-dependent species. In European water frogs, for instance, successful reproduction of a hybrid species (*R. esculenta,* genotype LR) requires mating with one of its parental species (*R. lessonae,* genotype LL), except in the rare cases where hybrids are triploid. The sexual host LL, however, should avoid matings with the sexual parasite LR, because the resulting LR offspring will eliminate the L genome from their germ line. In this study we investigate how this conflict is solved. Since water frog hybrids come in both sexes, rather than as females only like in other sperm-dependent systems, we performed the tests with both females and males. One individual was given a choice between two individuals of the opposite sex, one an LL and the other an LR. In both species, females showed the predicted preference for LL males, whereas males did not discriminate between LL and LR females. On the individual level, we interpret the sex difference in choosiness by the lower costs from mating with the wrong species (LR) and the higher benefits from mating with large individuals in males than in females. In "normal" species, male preference for large (i.e. more fecund) females is advantageous, but in this system such a choice can result in mating with the larger LR females. With respect to the structure and dynamics of mixed populations, we discuss that the observed female preference is consistent with the higher mating success of LL males found in nature. Hence, mate female choice is a strong candidate for a mechanism promoting coexistence of the sperm-dependent hybrid and its sexual host. This confirms predictions from previous theoretical models. *Key words:* coexistence, fitness, hybridogenesis, male competition, mate choice, population dynamics, reproductive success. *[Behav Ecol 12:600–606 (2001)]*

Traditionally, behavioral ecologists have studied mating patterns from the individual's point of view and have asked how mate choice and competition affect the fitness of females and males (reviewed by Andersson, 1994). In doing so, they have considered resource distribution, sex ratios, age structure, and other ecological and demographic conditions to explain why mating patterns differ so widely, both among and within species (e.g., Clutton-Brock, 1991; Davies, 1991; Emlen and Oring, 1977; Wittenberger, 1979). Only recently scientists have begun to ask the reverse question: How does individual behavior affect processes on higher levels, such as the composition and dynamics of populations and communities? (Fryxell and Lundberg, 1998; Sutherland, 1996). It is intuitively obvious that random mating—leading to "hybridizaiton" between individuals from different genotypes, families, populations, or species—can profoundly influence the population dynamics through changing fecundity, survival, and dispersal rates. It is also obvious that skewed reproductive success, resulting from individual differences in attractiveness and competitive abilities, will reduce the effective genetic and demographic population size, N_e, below the actual number, N (Caughley, 1994). The potential importance of this feedback from individual behavior to population biology has recently been highlighted with respect to conservation biology (Caro, 1998; Clemmons and Buchholz, 1997). Empirical evidence for actual effects, however, is extremely scarce, mainly because of the great complexity of interactions and the long time span between the observed behavior and its ecological consequences (Anholt, 1997). In this situation it may help to investigate systems with exceptionally fast and marked responses of the population to the behavior of its members.

Such systems exist in species with sperm-dependent reproduction. They require the sperm of other species for fertilizing their eggs (hybridogenesis) or for stimulating egg development (gynogenesis), but usually do not transmit the paternal genome to the next generation (reviewed by Beukeboom and Vrijenhoek, 1998; Dawley and Bogart, 1989). Such sexual parasites occur in a variety of invertebrate orders; among the Chordata, they are restricted to a few species of fishes and amphibians (Beukeboom and Vrijenhoek, 1998: Tables 2 and 3). At least in vertebrates, all sperm-dependent species seem to originally derive from natural hybridization between two sexual species (Arnold, 1997; Vrijenhoek, 1989) and have an initial demographic advantage over their sexual hosts, that is, the sperm donor species. This is either because the hybrids produce all-female offspring and, hence, save the two-fold costs of males (Maynard-Smith, 1978; Williams, 1975), or because their females are more fecund than the parental host females (Berger, 1977; Berger and Uzzell, 1980). With random mating, this should lead to instability and extinction of first the host and then the parasite (see below). In reality, however, such systems have been found to be remarkably sta-

ble over both ecological space (Berger, 1977; Moore, 1976) and evolutionary times (Hedges et al., 1992; Quattro et al., 1992; Spolsky et al., 1992).

In searching for the conditions under which such stability can be achieved, most theoretical models have focused on demographic and ecological mechanisms. The factors, which they have identified as crucial for a stable ratio between sexual and sperm-dependent species, include frequency-dependent mating success (Plötner and Grunwald, 1991), differences in female fecundity and offspring viability (Graf, 1986), some niche or microhabitat separation, strong asymmetric competition and/or a mildly biased sex ratio with <3–4 females/ male (Case and Taper, 1986; Guex et al., 1993; Kirkendall and Stenseth, 1990; Stenseth et al., 1985). But behavioral mechanisms can be equally effective. According to models by Moore and McKay (1971), Moore (1975), Som et al. (2000), and Hellriegel and Reyer (2000), movement between neighboring patches and discrimination between potential mates can stabilize local population dynamics, even when the ecological and demographic conditions are not fulfilled. In this study, we investigate whether the theoretically postulated mate choice does indeed occur, thus enabling a shift from random to assortative mating.

The hybridogenetic water frog complex

As a model system, we used a species complex of three central European water frogs: the pool frog (*R. lessonae*), the lake frog (*R. ridibunda*), and the edible frog (*R. esculenta*). *Rana esculenta* is originally a hybrid between the two other species (Berger, 1977), but differs from ordinary hybrids in many respects (see below). Hence, it is often referred to as a "species," too (see Günther, 1990 for a review of the nomenclature problem). Three features make this species complex unusual. First, in many parts of central Europe, including most of Switzerland, *R. ridibunda* (genotype RR) is absent from most areas, leaving mixed populations consisting of only *R. lessonae* (LL) and *R. esculenta* (LR). Second, *R. esculenta* has a reproductive mode, known as "hybridogenesis" (Schultz, 1969; Tunner, 1973, 1974). It eliminates the L genome from the germ line prior to meiosis, duplicates the remaining R genome and transmits it clonally (i.e., without recombination) to eggs and sperm. Thus, *R. esculenta* is a hemiclonal hybrid in terms of its phenotype, but *R. ridibunda* in terms of its clonal genetic contribution to the next generation. Third, hybrids come in both sexes, rather than as females only, like in other hybridogenetic and gynogenetic systems (Beukeboom and Vrijenhoek, 1998; Dawley and Bogart, 1989).

These features have important reproductive consequences (Figure 1). Homotypic matings between *R. lessonae* females and males (LL × LL) lead to *R. lessonae* offspring, whereas those between *R. esculenta* adults (LR × LR) result in *R. ridibunda* tadpoles; but the latter usually do not survive, probably due to an accumulation of deleterious mutations on the clonal R genome (Berger, 1976; Graf and Müller, 1979; Semlitsch and Reyer, 1992; Uzzell et al., 1980; for some rare exceptions—which include triploid populations—see Günther and Plötner, 1990; Hotz et al., 1992). As a consequence, *R. esculenta* will reproduce successfully only in mixed populations where they can mate with *R. lessonae* to regain the previously eliminated L genome. Both heterotypic mating combinations result in new *R. esculenta* animals, but the outcome differs in two important aspects. In terms of numbers, the combination *R. esculenta* female with *R. lessonae* male (LR × LL) produces 2–3 times as many offspring as the reverse combination (LL × LR), because hybrid LR females have a higher fecundity (Berger, 1977; Berger and Uzzell, 1980; Juszczyk, 1974, cited in Günther, 1990; Reyer et al., 1999). In terms of
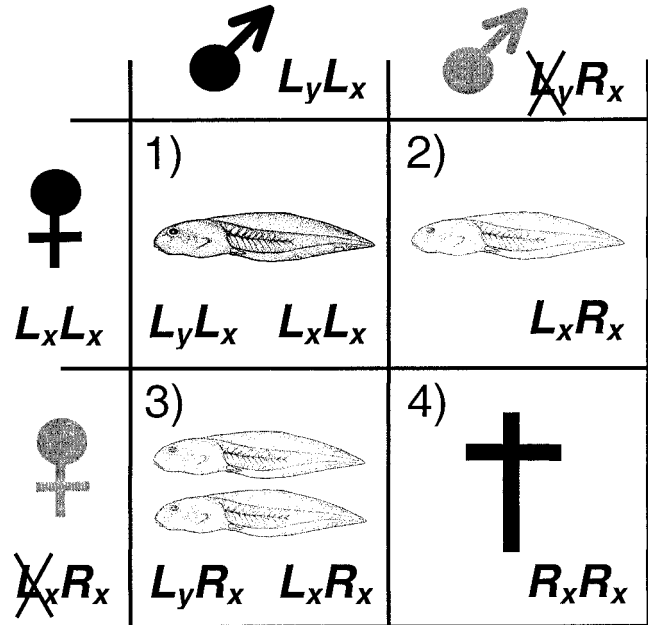

Figure 1
Possible mating combinations and resulting offspring (cells 1–4) in mixed populations of *R. lessonae* (genotype LL) and *R. esculenta* (genotype LR). L indicates that the hybrid *R. esculenta* eliminates the parental L genome premeiotically. Hence, it produces eggs and sperm, respectively, containing the R genome only. Since the hybrid's ridibunda genome is of maternal (R_x) and its *lessonae* genome of paternal origin (L_y), premeiotic elimination of the latter results in the exclusive production of R_x gametes. Consequently, matings with hybrid males (second column) lead to daughters only (L_xR_x, R_xR_x) whereas those with paternal males (first column) produce equal numbers of male (L_xL_x, L_yR_x) and female offspring (L_xL_x, L_xR_x). The higher number of tadpoles in cell 3 than in cells 1 and 2 illustrates the higher fecundity of LR compared to LL females; the cross in cell 4 indicates that these tadpoles do not survive to metamorphosis.

sex ratio, LR × LL usually leads to a 1:1 ratio among the offspring, where LL × LR normally results in all-daughter progeny (Berger et al., 1988; Hotz et al., 1992). The latter is due to the fact that—for size related reasons—primary hybridization probably occurred between LL males and RR females. Consequently, premeiotic exclusion of the L genome from the LR germ line usually affects the male genome with the consequence that male and female *R. esculenta* produce only gametes with the female genome (Figure 1).

In this hybridogenetic system, *R. esculenta* can be viewed as a sexual parasite who needs the parental species *R. lessonae* as a host to parasitize his genome every generation anew. *R. lessonae*, however, should avoid mating with hybrids, because the resulting LR offspring will in the next generation eliminate the parental L genome. Hence, there is a conflict between *R. lessonae* and *R. esculenta* over the best mating strategy. The outcome of this conflict not only affects the fitness of the individuals; it also strongly influences the structure and dynamics of the mixed populations. This is illustrated by the following three hypothetical scenarios (Figure 1). First, if mating were random, that is, proportional to the relative numbers of LL and LR males and females in the population, offspring would be produced in the ratio of 1 *R. lessonae* (cell 1 in Figure 1) to 3–4 *R. esculenta* (cells 2 and 3). Repeated over several years, this will dilute the proportion of the parental species to zero and then lead the hybrid population to extinction. Second, if all matings were heterotypic (cells 2 and

3), no *R. lessonae* offspring would be produced; hybrid numbers would first increase, but then collapse, because the sexual host is no longer available. In both scenarios hybrid daughters would outnumber hybrid sons by about 2:1. Such a surplus of hybrid females is, indeed, found in natural populations (Berger et al., 1988; Holenweg, 1999). Third, if matings were exclusively homotypic (cells 1 and 4) the *R. esculenta* would be doomed within one generation and a pure *R. lessonae* population would result. Thus, all three scenarios predict extinction, either of both species or of the hybrid alone.

This, however, is not what we observe in nature. Here, *R. lessonae/R. esculenta* ratios remain fairly stable over time within ponds, but differ between ponds (Berger, 1977; Blankenhorn, 1974, 1977; Holenweg, 1999). Recent theoretical models by Som et al. (2000) and Hellriegel and Reyer (2000) show that this temporal stability and spatial difference of species ratios is strongly influenced by the relative frequencies of the four possible mating combinations (Figure 1). These, in turn, can be expected to depend on the mate preferences of all four participants: LL females, LR females, LL males, and LR males. So far, rigorous choice experiments had been conducted with hybrid LR females alone; they revealed a significant preference for LL over LR males (Abt and Reyer, 1993) which is superimposed by male–male competition (Bergen et al., 1997). Two further studies, allegedly demonstrating a preference in males (Blankenhorn, 1974, 1977; Notter, 1974), have been criticized on the grounds of experimental flaws and a mismatch between results and interpretations (Abt and Reyer, 1993). The aim of this study was to fill the empirical gap and test the mate preferences of all four actors in this hybridogenetic mating system.

METHODS

Study site and animals
The experiments were performed between 30 April and 1 July 1993 on a military training ground, located close to the Zürich international airport at Kloten, Switzerland. All animals were captured at night from a nearby vegetated pond of about 60 m² surface area and 1 m depth. According to a mark-recapture study, its frog population numbered about 600 adults, with an LR/LL ratio of 35/65% (Reyer H-U and Abt G, unpublished data), which is typical for a pond of that size and type (Blankenhorn, 1977; Holenweg, 1999). All animals caught were weighed to nearest 1 g and measured with a precision of 1 mm (snout-vent length, SVL). Those smaller than 45 mm were immediately released back into the pond because they are unlikely to be sexually mature (Berger, 1970; Günther, 1990); those ≥ 45 mm were examined for species (LL or LR) and sex. An immediate species identification was based on phenotypic traits, including color, spot pattern, and the size and shape of the metatarsal tubercle (Berger, 1977; Günther, 1990), but this method is not fully reliable. Therefore, we also drew a small sample of lymph from an incision made into the web between two toes of a hind foot. The lymph was later subjected to albumin electrophoresis which allows unambiguous determination of the species (Tunner, 1973) and, hence, provided a check of the initial phenotypic assignment. Sex was determined from the presence (male) or absence (female) of vocal sacs and thumb pads. To ensure sexual interest we kept only males ≥ 45 mm, which—without exception—all had swollen thumb pads. Females were only kept when swollen with eggs and/or caught in amplexus without emitting a release call.

Between this catching and handling procedure and the start of the choice experiment, all frogs were kept in cages (1.5 × 1 × 0.5 m) for a few hours to several days, separated by sex and species, and individually marked with numbered
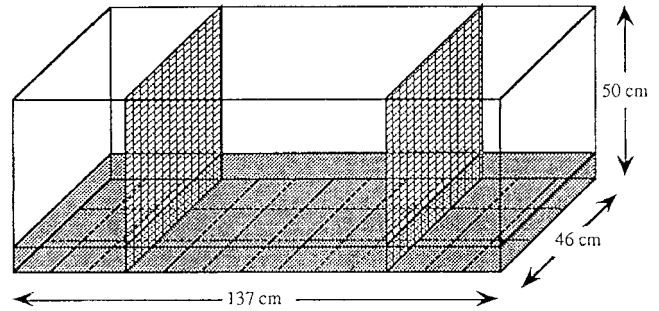

Figure 2
Test arena for mate choice experiments. A grid divides the length of the tank into 13 sectors of 10.5 cm each and the depth into 3 sectors of 15.3 cm each. Two wire screens (mesh size 1 × 1 cm) separate a 7-sector wide central compartment for the test animal from two 3-sector wide distal compartments holding one target individual each. The test frog was considered to be with the target frog when it stayed within the sector adjacent to the partitions.

waist bands (Emlen, 1968). These cages were placed at the shore of another pond in such a way that the animals had access to both water and land. After having completed its choice experiment, each frog was freed from the waist band and released back into his home pond, but only after marking him with an incision into one foot web to avoid repeated use of the same individual.

Experimental setup
The test arena consisted of a Plexiglas tank, filled with water to a level of 7 cm (Figure 2). A grid below the tank divided its bottom into 13 × 3 sectors (length × depth). Two wire screens separated a central compartment with 7 × 3 sectors from two distal ones with 3 × 3 sectors each. For a choice experiment, three frogs were transferred from the holding pens into this arena. One test animal (either female or male) was placed into a small wire cage (20 × 40 × 25 cm) in the middle of the central compartment, and two target animals of the opposite sex (one LL, one LR) were put into the distal compartments. After 5 min of acclimation, the wire cage was lifted and the test animal allowed to move freely in the central compartment for 30 min. Thereafter each of the LL and LR target animals were moved to the compartment on the other side to compensate for potential side preferences, and the procedure was repeated. At the end of this second 30-min session, all three frogs were removed from the arena, and the experiment was repeated with another set of three frogs. In order to avoid that potential chemical cues from one experiment carry over to the next, the water in the tank was stirred between the two 30-min sessions of an experiment and it was changed between two experiments. During both the acclimation and the actual choice period, the frogs were stimulated using a tape with a mixed chorus of LL and LR calls, occasionally joined in by real frogs from a pond some 30 m away. While each test animal was used only once, some target frogs served in more than one experiment, but each time in a different combination of individuals. Within this restriction, test and target frogs were selected randomly from the holding pens.

Variables and statistics
From a car, parked about 1.5 m off the test arena, we recorded every 30 s the following variables for all three animals:

- Position: whereabouts of the frog within the grid of the tank bottom.
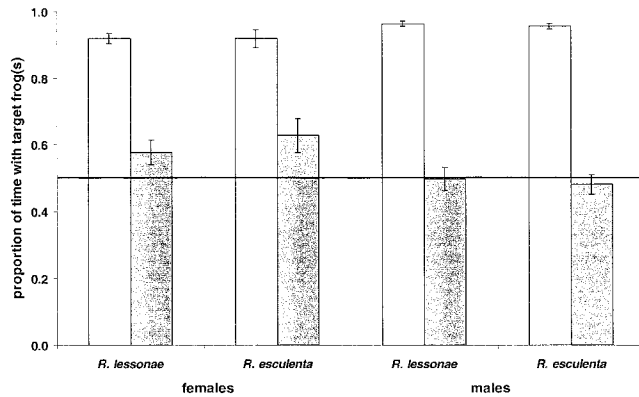- Activity: a change from one grid cell to another since the last position was recorded.

Figure 3
Proportion of time which the test frogs spent with either of the 2 target frogs and with *R. lessonae*. Total proportion (open bars) is expressed in relation to the total observation time of 60 min, the proportion spent with *R. lessonae* (shaded) in relation to the total time spent with either target frog. Shown are means and standard errors. The solid horizontal line indicates the expected proportion of time spent with *R. lessonae*, assuming no preferences, that is, a random distribution between LL and LR target individuals.

- Climbing: the presence or absence of climbing movements at the wire screen, which indicate an attempt to closely approach the target animal behind the partition.
- Calling: vocalization of males (yes/no) during a 30-s period.

Since calling was extremely rare, it was not further considered in the analyses. For the other three variables, data recorded for all 120 of the 30-s intervals that constitute an experiment (60 from each of the two 30-min sessions) were pooled to yield measures for the individuals' total amount of *activity* and the *time* spent in various sectors of their compartments. *Time* was calculated by multiplying the scan interval of 30 s by the number of recordings. The test animal's *interest* in the target frogs was measured by comparing the *time* it spent in the two sectors closest to the partition (Figure 2) against a random distribution. *Preference* was expressed as the difference between the *times* spent in the sectors adjacent to the LL and LR target frogs, respectively, and by the difference in *climbing* directed towards them. Based on expectations from hybridogenetic reproduction (see introduction) and from previous empirical results (Abt and Reyer, 1993), the alternative to the null hypothesis of no preference was a preference for LL individuals. Hence, the region of rejection was only at one end of the sampling distribution, which called for

a one-tailed test. Since *R. lessonae* and *R. esculenta* differ in average size and activity (Blankenhorn, 1974; Günther, 1990), and these variables are known or suspected to affect mate choice, even within species (e.g., Howard, 1988; Marquez, 1993), we further tested whether *preference* was related to *activity differences* between the two target animals and to *body size,* that is, to the difference in the SVL of the two target animals.

RESULTS

We tested a total of 97 frogs, but had to discard data from 62 individuals for the following reasons: (1) the (probably scared) test animal remained motionless for more than 50% of the observation period ($n = 21$); (2) the test animal never changed tank sides to inspect both target frogs at least once ($n = 28$); (3) albumin electrophoresis corrected the initial species assignment based on phenotype and revealed that both target frogs had been of the same species ($n = 10$) or one was a *R. ridibunda* ($n = 1$); (4) by mistake an individual was tested twice ($n = 1$); and (5) the test frog escaped from the arena during the experiment ($n = 1$). Criteria (1) and (2) had been defined a priori, whereas criteria (3) to (5) emerged only during the experiments and analyses, respectively. This left us with data from 35 frogs (11 LR females, 7 LL females, 7 LR males, and 10 LL males) which moved between the opposite ends of the test arena and clearly inspected both target animals.

Figure 3 shows that test animals of both species and sexes spent significantly more time in the two compartment sectors bordering the wire partitions than expected by chance. This is true, no matter whether expectation is calculated from the number of sectors (0.28) or from the total length of the central compartment's edges (0.56) which the animals preferred (all $p < .001$; range of t values: 13.03–84.19, range of df: 6–10; two-tailed t tests for pairwise comparisons between observed and expected times). When total time near partitions is broken down by species of the target frogs, it turns out that test animals of both species behaved in the same way (Figure 3): females spent significantly more time with LL than with LR males (both $p < .05$; *R. esculenta*, $t = -2.442$, df = 10, *R. lessonae*, $t = -2.073$, df = 6; one-tailed t tests for pairwise comparisons between observed and expected times) whereas males showed no preference for either LL or LR females (both $p \geq .528$; *R. esculenta*, $t = 0.669$, df = 6, *R. lessonae*, $t = 0.124$, df = 9).

A more detailed analysis (MANOVA, Table 1) which included the location of the target frogs (left or right compartment) as well as activity and size differences between them con-

Table 1

Differences (LL-LR) in time spent with target frogs and in climbing at their partitions

Dependent variables Independent variables	Multivariate test			Univariate tests				
					Time		Climbing	
	df	F (Wilks' λ)	p	df	F	p	F	p
Species	2, 26	1.111	.344	1, 27	0.659	.424	0.197	.661
Sex	2, 26*	4.605*	.019*	1, 27*	9.316*	.005*	5.608*	.025*
Species × sex	2, 26	0.620	.546	1, 27	1.157	.292	0.183	.672
Side of target frog	2, 26	0.544	.587	1, 27	1.022	.321	0.168	.685
Activity difference	2, 26	1.197	.318	1, 27	0.045	.833	1.751	.197
Size difference	2, 26	1.676	.207	1, 27	0.092	.764	1.435	.241

Results are from a MANOVA relating test animal factors (species, sex, and species × sex interaction) to target frog covariates (side of the arena where placed, activity differences, and size differences). Shown are df, *F,* and *p* values for multivariate and univariate tests.
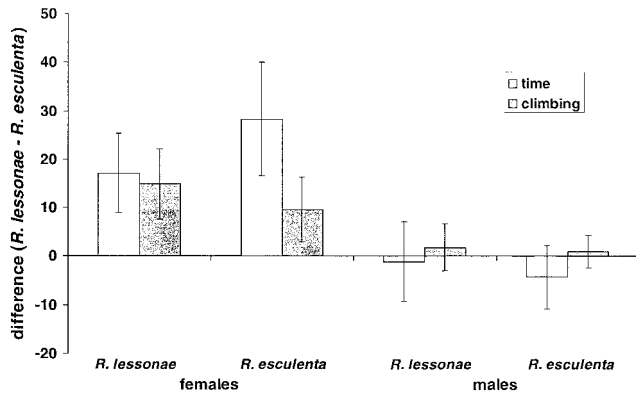* Significant results.

Figure 4
Differences in time spent (open bars) and frequency of climbing (shaded bars) at the partitions of LL and LR target individuals, respectively. Positive values indicate a preference for *R. lessonae*. For significance, see Table 1.

firmed and extended the above result: both the time spent with the target frogs and the frequency of climbing at the partitions is independent of the test animal's species, but differs between the sexes (Figure 4). While males did not discriminate between females of the two species, females spent significantly more time near *R. lessonae* males and climbed more at the partition separating them. The analysis also showed that this female preference for LL males could not be explained through differences in the target males' activity and/or body size (Table 1). This, and the fact that target animals almost never vocalized, suggests that females can choose LL males by their phenotype, independent of their behavior and size.

DISCUSSION

Causes for the observed sex differences in mate choice

Our experiments show the same behavior in both the parental species and the hybrid: where males do not discriminate between females of the two species, females prefer *R. lessonae* to *R. esculenta* males. Since males hardly ever called during the experiments, and size and activity did not affect the choice, female preferences must have been based on other cues. Without knowledge of the nature of these cues it is futile to look for proximate mechanisms as potential reasons for the observed sex differences. Below, we discuss two (not mutually exclusive) ultimate reasons, why females and males differ in their choosiness.

Costs of mating with the wrong species
The consequences of mating with a hybrid are the same for both sexes: no genetic contribution to the next generation, either because the offspring are not viable (LR × LR matings) or because they exclude the L genome when sexually mature (LR × LL and LL × LR matings; see introduction and Figure 1). However, the lifetime fitness cost of such a reproductive failure is likely to be higher in females than in males. Females usually spawn only once per season (Günther, 1990) and, hence, lose the reproductive effort of a whole year, where males stay at a pond for several weeks and can mate repeatedly (Abt and Reyer, 1993; Günther, 1990; Schuchardt and Klingel, 1984). Moreover, water frogs have a strongly skewed operational sex ratio (OSR) with males outnumbering females; this is typical for prolonged breeders in anurans (Wells, 1977). As a result, females usually have at least the theoretical option of choosing the preferred LL males, where for males unpaired

LL females are often not available. In such a situation, the costs of erroneously amplexing an LR female may be low, compared to the costs of discriminating between females of the two species and to the benefits from choosing large females, which are discussed in the following paragraph.

Benefits of mating with the right size
For mechanical reasons, optimal fertilization success requires size-assortative mating, that is, female/male size ratios which are not too extreme (Davies and Halliday, 1977; Gerhardt et al., 1987; Robertson, 1990; Ryan, 1985). Within the suitable size range, however, selection will act on males to prefer large females of higher fecundity (Blankenhorn, 1974, 1977; Notter, 1974) and on females to chose smaller or at most equal-sized males, because this will ease swimming and spawning (Licht, 1976; Robertson, 1986). Since, on average, *R. esculenta* is bigger than *R. lessonae*, size cues alone should direct males toward hybrid females, but females towards parental males. Thus, for females, both the benefits from mating with the right size and costs from mating with the (genetically) wrong species predict the observed preference for LL males. In males, however, the genetically beneficial choice of LL females is opposed by a size-related preference for LR females. This may explain their indiscriminate behavior.

Such erroneous and futile matings, resulting from responses to simple fertility indicators, have also been demonstrated for males of the fishes *Poecilia mexicana* and *P. latipinna*: although able to recognize their respective conspecific females (Hubbs, 1964; Ryan et al., 1996; Schlupp and Ryan, 1996), they prefer receptive hybrid females of the gynogenetic *P. formosa* over nonreceptive females of their own sexual species (Schlupp et al., 1991). Whether choice is mainly based on a single open-ended trait, indicating mate quality, or is modified by other cues, will depend on the likelihood of making a mistake in recognition and the fitness costs of mating with heterospecifics (Pfennig, 1998). In gray tree frogs (*Hyla chrysoscelis*) and spadefoot toads (*Spea multiplicata*), for instance, females from populations overlapping with congeners weigh species identifying call properties more heavily than properties indicating mate quality, whereas those from allopatric populations do not (Gerhardt, 1994; Pfennig, 2000). Since, at least in gray tree frogs, properties of male calls do not differ between sympatric and allopatric populations, this not only indicates a shift in trade-off from quality to species discrimination with increasing risk of hybridization; it also supports the notion that females have more to lose than males (Gerhardt, 1994). In this respect, it would be interesting to compare the mate choice of female and male water frogs from populations with low and high proportions of hybrids and different sex ratios.

Mate choice and mating in nature

How relevant are the side associations measured in our study for mate choice and mating patterns in nature? In anurans, male vocalization plays a predominant role in attracting females; consequently, studies of female choice almost exclusively use phonotactic approaches to measure it. However, anecdotal observations and experimental evidence suggest that movements towards and away from (even noncalling) males as well as temporal changes in next-neighbor distances also reflect sexual interest and are used to compare among different potential mates (Abt and Reyer, 1993; Bergen et al., 1997; Blankenhorn, 1974, 1977; Reyer H-U and Frei G, unpublished data; Roithmair, 1994). Moreover, the female preference for *R. lessonae* over *R. esculenta* males found in our experiment is consistent with results from other studies, indicating that LL males are more successful in reproduction than LR males.

In an experiment measuring the combined effects of female choice and male-male competition on actual mating combinations, Bergen et al. (1997) found males to be successful in a ratio of 57% LL:43% LR. This is close to the 60:40% time ratio in favor of LL males that we found (Figure 3) and the 66:44% ratio among fertilized egg masses found in a natural pond, where 51% of all clutches originated from the LL × LL mating combination (Abt G, unpublished data; see also Blankenhorn, 1977; Radwan and Schneider, 1988). In other natural ponds, females were found in amplexus with LL and LR males, respectively, even in the ratio of 81:19% (Reyer H-U, unpublished data). These latter ratios, which are already corrected for expectations from random mating, suggest the existence of additional mechanisms which skew the success of LL males beyond the preference of 60:40% found in this study.

Potential candidates for such mechanisms include: (1) approach to aggregations of preferred males from some distance by using their mating calls for orientation (Roesli and Reyer, 2000); (2) avoidance of fast movements, direct contact, and other cues which normally stimulate the indiscriminate males to forcefully amplex (Bourne, 1992; Emlen, 1976; Grüsser and Butenandt, 1968; Notter, 1974; Robertson, 1986; Ryan, 1985; and our own observations), (3) vertical body positions, release calls and provoking of fights to get rid of amplectant males (Abt and Reyer, 1993; Blankenhorn, 1977), (4) "cryptic" choice through reducing the clutch size when spawning with an LR male (Reyer et al., 1999), and (5) possibly a male trait, rather than a female preference. In this respect, however, the evidence is controversial. Blankenhorn (1974, 1977) suggested that *R. lessonae* males gained more mates because of their appropriate sexual, rather than aggressive, behavior in the presence of females, where Bergen et al. (1997) concluded that *R. esculenta* males were relatively successful in achieving matings, because they showed high levels of aggression against other males, including competing *R. lessonae*. Ongoing experiments suggest that the competitive ability of males may vary with the LL/LR ratio (Reyer H-U, unpublished data).

Whatever the precise mechanism, the female preference for LL males found in this and other studies (Abt and Reyer, 1993; Reyer et al., 1999; Roesli and Reyer, 2000) is consistent with the fact that in mixed populations of *R. lessonae* and *R. esculenta* the relative frequencies of the four possible mating combinations (LL × LL, LL × LR, LR × LL, LR × LR) are shifted from those expected under the assumption of random mating to those involving LL males. This assortative mating pattern results in a reduced number of LR offspring, which is crucial for promoting coexistence of the sperm-dependent hybrid and its sexual host (Hellriegel and Reyer, 2000; Som et al., 2000). However, further studies are needed to answer the question how mating behavior affects the population dynamics in detail, especially whether and how it also contributes to the markedly different LL/LR ratios found in natural ponds (Berger, 1977; Blankenhorn, 1974, 1977; Holenweg, 1999). These investigations are presently under way.

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.

Andersson M, 1994. Sexual selection. Princeton, New Jersey: Princeton University Press.

Anholt BR, 1997. How should we test for the role of behaviour in population dynamics? Evol Ecol 11:633–640.

Arnold ML, 1997. Natural hybridization and evolution. Oxford: Oxford University Press.

Bergen K, Semlitsch RD, 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, 1970. Some characteristics of the crosses within *Rana esculenta* complex in post larval development. Annl Zool Warsz 27:373–416.

Berger L, 1976. Hybrids of B-2 generations of European water frogs (*Rana esculenta* complex). Annl Zool Warsz 33:201–214.

Berger L, 1977. Systematics and hybridization in the *Rana esculenta* complex. In: The reproductive biology of amphibians (Taylor DH, Guttmann SI, eds). New York: Plenum Press; 367–388.

Berger L, Uzzell T, 1980. The eggs of European water frogs of the *Rana esculenta*-complex and their hybrids. Folia Biol 28:3–26.

Berger L, Uzzell T, Hotz H, 1988. Sex determination and sex ratios in western Palearctic water frogs: XX and XY female hybrids in the Pannonian basin? Proc Acad Nat Sci Phila 140:220–239.

Beukeboom LW, Vrijenhoek RC, 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. J Evol Biol 11:755–782.

Blankenhorn H, 1974. Soziale organisation einer mischpopulation von *Rana lessonae* camerano und *Rana esculenta* linnaeus (Ph.D. thesis). Zürich: University of Zürich.

Blankenhorn H, 1977. Reproduction and mating behaviour in *Rana lessonae-Rana esculenta* mixed populations. In: The reproductive biology of amphibians (Taylor DH, Guttman SI, eds). New York: Plenum Press; 389–410.

Bourne GR, 1992. Lekking behavior in the neotropical frog *Ololygon rubra*. Behav Ecol Sociobiol 31:173–180.

Caro T, 1998. Behavioral ecology and conservation biology. Oxford: Oxford University Press.

Case TJ, Taper ML, 1986. On the coexistence and coevolution of asexual and sexual competitors. Evolution 40:366–387.

Caughley G, 1994. Directions in conservation biology. J Anim Ecol 63:215–244.

Clemmons JR, Buchholz R, 1997. Behavioral approaches to conservation in the wild. Cambridge: Cambridge University Press.

Clutton-Brock TH, 1991. The evolution of parental care. Princeton, New Jersey: Princeton University Press.

Davies NB, 1991. Mating systems. In: Behavioural ecology—an evolutionary approach, 3rd ed. (Krebs JR, Davies NB, eds). Oxford: Blackwell Scientific Publications; 263–294.

Davies NB, Halliday TR, 1977. Optimal mate selection in the toad *Bufo bufo*. Nature 269:56–58.

Dawley RM, Bogart JP, 1989. Evolution and ecology of unisexual vertebrates. Bulletin 466. Albany, New York: The New York State Museum.

Emlen ST, 1968. A technique for marking anuran amphibians for behavioral studies. Herpetologica 24:172–173.

Emlen ST, 1976. Lek organization and mating strategies in the bullfrog. Behav Ecol Sociobiol 1:283–313.

Emlen ST, Oring LW, 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223.

Fryxell JM, Lundberg P, 1998. Individual behavior and community dynamics. London: Chapman & Hall.

Gerhardt HC, 1994. Reproductive character displacement of female mate choice in the grey tree frog *Hyla chrysoscelis*. Anim Behav 47:959–969.

Gerhardt HC, Daniel RE, Perrill SA, Schramm S, 1987. Mating behavior and male mating success in the green tree frog. Anim Behav 35:1490–1503.

Graf J-D, 1986. Population genetics of the *Rana esculenta* complex: A model. In: Studies in herpetology (Rocek Z, ed). Prague: Charles University; 175–180.

Graf J-D, Mueller WP, 1979. Experimental gynogenesis provides evi-

dence of hybridogenetic reproduction in the *Rana esculenta* complex. Experientia 35:1574–1576.

Grüsser OJ, Butenandt E, 1968. The effect of stimulus on the response of movement detecting neurons in the frog retina. Pflügers Archiv 298:283–293.

Günther R, 1990. Die wasserfrösche Europas (Anuren-Froschlurche). Wittenberg: Neue Brehm Bücherei.

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

Guex GD, Beerli P, Barbour AD, Hotz H, 1993. A dynamic model to describe equilibrium conditions in mixed populations of a hemiclonal hybrid and its sexual host in European water frogs. 4th Congress European Society for Evolutionary Biology: 158.

Hedges SB, Bogart JP, Maxson LR, 1992. Ancestry of unisexual salamanders. Nature 356:708–710.

Hellriegel B, 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.

Holenweg A-K, 1999. Dispersal and population dynamics in water frogs, *Rana lessonae, R. ridibunda* and their hybridogenetic associate *R. esculenta* (Ph.D. thesis). Zürich: University of Zürich.

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.

Howard RD, 1988. Sexual selection on male body size and mating behaviour in American toads, *Bufo americanus*. Anim Behav 36: 1796–1808.

Hubbs C, 1964. Interactions between bisexual fish species and its gynogenetic sexual parasite. Bull Tex Mem Mus 8:1–72.

Kirkendall LR, Stenseth NC, 1990. Ecological and evolutionary stability of sperm-dependent parthenogenesis: effects of partial niche overlap between sexual and asexual females. Evolution 44:698–714.

Licht LE, 1976. Sexual selection in toads (*Bufo americanus*). Can J Zool 54:1277–1284.

Marquez R, 1993. Male reproductive success in 2 midwife toads, *Alytes obstetricans* and A. *cisternasii*. Behav Ecol Sociobiol 32:283–291.

Maynard-Smith J, 1978. The evolution of sex. Cambridge: Cambridge University Press.

Moore WS, 1976. Components of fitness in the unisexual fish *Poeciliopsis monacha-occidentalis*. Evolution 30:564–578.

Moore WS, McKay FE, 1971. Coexistence in unisexual-bisexual species complexes of *Poeciliopsis* (Pisces: Poeciliidae). Ecology 52:791–799.

Notter P, 1974. Zum paarungsverhalten von *Rana lessonae* Cam. und *Rana esculenta* L (M.Sc. thesis). Zürich: University of Zürich.

Pfennig KS, 1998. The evolution of mate choice and the potential for conflict between species and mate-quality recognition. Proc R Soc Lond B 265:1743–1747.

Pfennig KS, 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. Behav Ecol 11:220–227.

Plötner L, Grunwald C, 1991. A mathematical model of the structure and the dynamics of *Rana ridibunda/esculenta*-male populations (Anura, Ranidae). Z Zool Syst Evol-forsch 29:201–207.

Quattro JM, Avise JC, Vrijenhoek RC, 1992. An ancient clonal lineage in the fish genus *Poeciliopsis* (Atheriniformes, Poeciliidae). Proc Natl Acad Sci USA 89:348–352.

Radwan MM, Schneider H, 1988. Social behaviour, call repertory and variation in the calls of the pool frog, *Rana lessonae* (Anura: Ranidae). Amphibia-Reptilia 9:329–351.

Reyer H-U, Frei G, Som C, 1999. Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. Proc R Soc Lond B 266:2101–2107.

Robertson JGM, 1986. Female choice, male strategies and the role of vocalization in the Australian frog *Uperoleia rugosa*. Anim Behav 34: 773–784.

Robertson JGM, 1990. Female choice increases fertilization success in the Australian frog, *Uperoleia laevigata*. Anim Behav 39:639–645.

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

Roithmair ME, 1994. Male territoriality and female mate selection in the dart-poison frog *Epipedobates triviottatus* (Dendrobatidae, Anura). Copeia 1994:107–115.

Ryan MJ, 1985. The Túngara frog. Chicago: University of Chicago Press.

Ryan MJ, Dries LA, Batra P, Hillis DM, 1996. Male mate preferences in a gynogenetic species complex of Amazon mollies. Anim Behav 52:1225–1236.

Schlupp I, Parzefall J, Schartl M, 1991. Male mate choice in mixed bisexual/unisexual breeding complexes of *Poecilia* (Teleostei: Poeciliidae). Ethology 88:215–222.

Schlupp I, Ryan MJ, 1996. Mixed-species shoals and the maintenance of a sexual-asexual mating system in mollies. Anim Behav 52:885–890.

Schuchardt H-G, Klingel H, 1984. Sozialverhalten einheimischer Grünfrösche. Verh Dtsch Zool Ges 77:328.

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

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

Som C, Anholt BR, 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.

Spolsky CM, Phillips CA, Uzzell T, 1992. Antiquity of clonal salamander lineages revealed by mitochondrial DNA. Nature 356:706–708.

Stenseth NC, Kirkendall LR, Moran N, 1985. On the evolution of pseudogamy. Evolution 39:294–307.

Sutherland WJ, 1996. From individual behaviour to population ecology. Oxford: Oxford University Press.

Tunner HG, 1973. Das Albumin und andere Bluteiweisse bei *Rana ridibunda* Pallas, *Rana lessonae* Camerano, *Rana esculenta* Linné und deren Hybriden. Z Zool Syst Evol-forsch 11:219–233.

Tunner HG, 1974. Die klonale Struktur einer Wasserfroschpopulation. Z Zool Syst Evol-forsch 12:309–314.

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.

Vrijenhoek RC, 1989. Genotypic diversity and coexistence among sexual and clonal lineages of *Poeciliopsis*. In: Speciation and its Consequences (Otte D, Endler J, eds). Sunderland: Sinauer; 386–400.

Wells KD, 1977. The social behaviour of anuran amphibians. Anim Behav 25:666–693.

Williams GC, 1975. Sex and evolution. Princeton, New Jersey: Princeton University Press.

Wittenberger JF, 1979. The evolution of mating systems in birds and mammals. In: Handbook of behavioral neurobiology: social behavior and communication (Master P, Vandenburgh J, eds). New York: Plenum Press; 271–349.