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Abstract

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.

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

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

1 Traditionally, behavioral ecologists have studied mating patterns from the individual's
2 point of view and have asked how mate choice and competition affect the fitness of
3 females and males (reviewed by Andersson 1994). In doing so, they have considered
4 resource distribution, sex ratios, age structure and other ecological and demographic
5 conditions to explain why mating patterns differ so widely, both among and within
6 species (e.g. Clutton-Brock, 1991; Davies, 1991; Emlen and Oring, 1977;
7 Wittenberger, 1979). Only recently scientists have begun to ask the reverse question:
8 How does individual behavior affect processes on higher levels, such as the
9 composition and dynamics of populations and communities? (Fryxell and Lundberg,
10 1998; Sutherland, 1996). It is intuitively obvious that random mating – leading to
11 “hybridization” between individuals from different genotypes, families, populations or
12 species - can profoundly influence the population dynamics through changing
13 fecundity, survival and dispersal rates. It is also obvious that skewed reproductive
14 success - resulting from individual differences in attractiveness and competitive
15 abilities - will reduce the effective genetic and demographic population size (N_e)
16 below the actual number (N) (Caughley, 1994). The potential importance of these
17 feedbacks from individual behavior to population biology has recently been
18 highlighted with respect to conservation biology (Caro, 1998; Clemmons and
19 Buchholz, 1997). Empirical evidence for actual effects, however, is extremely scarce,
20 mainly because of the great complexity of interactions and the long time span
21 between the observed behavior and its ecological consequences (cf. Anholt, 1997).
22 In this situation it may help to investigate systems with exceptionally fast and marked
23 responses of the population to the behavior of its members.

24 Such systems exist in species with sperm-dependent reproduction. They
25 require the sperm of other species for fertilizing their eggs (“hybridogenesis”) or for
26 stimulating egg development (“gynogenesis”), but usually do not transmit the paternal
27 genome to the next generation (reviewed by Dawley and Bogart, 1989; Beukeboom
28 and Vrijenhoek, 1998). Such sexual parasites occur in a variety of invertebrate
29 orders; among the Chordata, they are restricted to a few species of fishes and
30 amphibians (see Tables 2 and 3 in Beukeboom and Vrijenhoek, 1998). At least in
31 vertebrates, all sperm-dependent species seem to originally derive from natural
32 hybridization between two sexual species (Vrijenhoek, 1989; Arnold, 1997) and have
33 an initial demographic advantage over their sexual hosts, i.e. the sperm-donor
34 species. This is either because the hybrids produce all-female offspring and, hence,

1 save the two-fold costs of males (Williams, 1975; Maynard-Smith, 1978), or because
2 their females are more fecund than the parental host females (Berger 1977; Berger
3 and Uzzell, 1980). With random mating, this should lead to instability and extinction
4 of first the host and then the parasite (see below). In reality, however, such systems
5 have been found to be remarkably stable over both ecological space (Moore, 1976;
6 Berger, 1977) and evolutionary times (Hedges et al., 1992; Quattro et al., 1992;
7 Spolsky et al., 1992).

8 In searching for the conditions under which such stability can be achieved,
9 most theoretical models have focussed on demographic and ecological mechanisms.
10 The factors, which they have identified as crucial for a stable ratio between sexual
11 and sperm-dependent species, include frequency-dependent mating success
12 (Plötner and Grunwald, 1991), differences in female fecundity and offspring viability
13 (Graf, 1986), some niche or microhabitat separation, strong asymmetric competition
14 and/or a mildly biased sex ratio with <3-4 females/male (Stenseth et al., 1985; Case
15 and Taper, 1986; Kirkendall and Stenseth, 1990; Guex et al., 1993). But behavioral
16 mechanisms can be equally effective. According to models by Moore and McKay
17 (1971), Moore (1975), Som et al. (2000) and Hellriegel and Reyer (2000), movement
18 between neighboring patches and discrimination between potential mates can
19 stabilize local population dynamics, even when the ecological and demographic
20 conditions are not fulfilled. In this study, we investigate whether the theoretically
21 postulated mate choice does indeed occur, thus enabling a shift from random to
22 assortative mating.

23

24 **The hybridogenetic water frog complex**

25

26 As a model system, we used a species complex of three central European water
27 frogs: the pool frog (*R. lessonae*), the lake frog (*R. ridibunda*) and the edible frog (*R.*
28 *esculenta*). *Rana esculenta* is originally a hybrid between the two other species
29 (Berger 1977), but differs from ordinary hybrids in many respects (see below).
30 Hence, it is often referred to as a “species”, too (see Günther 1990 for a review of the
31 nomenclature problem). Three features make this species complex unusual. First, in
32 many parts of central Europe, including most areas of Switzerland, *R. ridibunda*
33 (genotype RR) is absent from most areas, leaving mixed populations consisting of
34 only *R. lessonae* (LL) and *R. esculenta* (LR). Second, *R. esculenta* has a
35 reproductive mode, known as “hybridogenesis” (Schultz, 1969; Tunner, 1973, 1974).

1 It eliminates the L-genome from the germ line prior to meiosis, duplicates the
2 remaining R-genome and transmits it clonally (i.e. without recombination) to eggs
3 and sperm. Thus, *R. esculenta* is a hemiclonal hybrid in terms of its phenotype, but a
4 *R. ridibunda* in terms of his clonal genetic contribution to the next generation. Third,
5 hybrids come in both sexes, rather than as females only like in other hybridogenetic
6 and gynogenetic systems (Dawley and Bogart, 1989; Beukeboom and Vrijenhoek,
7 1998).

8 These features have important reproductive consequences (Figure 1).
9 Homotypic matings between *R. lessonae* females and males (LL x LL) lead to *R.*
10 *lessonae* offspring, whereas those between *R. esculenta* adults (LR x LR) result in *R.*
11 *ridibunda* tadpoles; but the latter usually do not survive, probably due to an
12 accumulation of deleterious mutations on the clonal R-genome (Berger, 1976; Graf
13 and Müller, 1979; Semlitsch and Reyer, 1992; Uzzell et al., 1980; for some rare
14 exceptions – which include triploid populations – see Günther and Plötner, 1990;
15 Hotz et al., 1992). As a consequence, *R. esculenta* will reproduce successfully only
16 in mixed populations where they can mate with *R. lessonae* to regain the previously
17 eliminated L-genome. Both heterotypic mating combinations result in new *R.*
18 *esculenta* animals, but the outcome differs in two important aspects. In terms of
19 numbers, the combination *R. esculenta* female with *R. lessonae* male (LR x LL)
20 produces 2-3 times as many offspring as the reverse combination (LL x LR), because
21 hybrid LR-females have a higher fecundity (Berger, 1977; Berger and Uzzell, 1980;
22 Juszczuk, 1974, cited in Günther, 1990; Reyer et al., 1999). In terms of sex ratio, LR
23 x LL usually leads to a 1:1 ratio among the offspring, whereas LL x LR normally
24 results in all-daughter progeny (Berger et al., 1988; Hotz et al., 1992). The latter is
25 due to the fact that – for size related reasons – primary hybridization probably
26 occurred between LL-males and RR-females. Consequently, premeiotic exclusion of
27 the L-genome from the LR germ line usually affects the male genome with the
28 consequence that male and female *R. esculenta* produce only gametes with the
29 female genome (cf. Figure 1).

30 **Insert Figure 1 about here**

31
32 In this hybridogenetic system, *R. esculenta* can be viewed as a sexual
33 parasite who needs the parental species *R. lessonae* as a host to parasitize his
34 genome every generation anew. *R. lessonae*, however, should avoid mating with

1 hybrids, because the resulting LR-offspring will in the next generation eliminate the
2 parental L-genome. Hence, there is a conflict between *R. lessonae* and *R. esculenta*
3 over the best mating strategy. The outcome of this conflict not only affects the fitness
4 of the individuals; it also strongly influences the structure and dynamics of the mixed
5 populations. This is illustrated by the following three hypothetical scenarios (cf.
6 Figure 1). First, if mating were random, i.e. proportional to the relative numbers of
7 LL- and LR- males and females in the population, offspring would be produced in the
8 ratio of 1 *R. lessonae* (cell 1 in Figure 1) to 3-4 *R. esculenta* (cells 2 and 3).
9 Repeated over several years, this will dilute the proportion of the parental species to
10 zero and then lead the hybrid population to extinction. Second, if all matings were
11 heterotypic (cells 2 and 3), no *R. lessonae* offspring would be produced; hybrid
12 numbers would first increase, but then collapse, because the sexual host is no longer
13 available. In both scenarios hybrid daughters would outnumber hybrid sons by about
14 2:1. Such a surplus of hybrid females is, indeed, found in natural populations (Berger
15 et al., 1988; Holenweg, 1999). Third, if matings were exclusively homotypic (cells 1
16 and 4) the *R. esculenta* would be doomed within one generation and a pure *R.*
17 *lessonae* population would result. Thus, all three scenarios predict extinction, either
18 of both species or of the hybrid alone.

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

1 MATERIAL AND METHODS

2

3 Study site and animals

4

5 The experiments were performed between April 30th and July 1st 1993 on a military
6 training ground, located close to the Zürich international airport at Kloten. All animals
7 were captured at night from a nearby vegetated pond of about 60 m² surface area
8 and 1 m depth. According to a mark-recapture study, its frog population numbered
9 about 600 adults, with a LR/LL-ratio of 35/65 % (Reyer and Abt, unpubl. data), which
10 is typical for a pond of that size and type (Blankenhorn, 1977; Holenweg, 1999). All
11 animals caught were weighed to nearest 1 g and measured with a precision of 1 mm
12 (snout-vent-length, SVL). Those smaller than 45 mm were immediately released
13 back into the pond because they are unlikely to be sexually mature (Berger, 1970;
14 Günther, 1990); those ≥ 45 mm were examined for species (LL or LR) and sex. An
15 immediate species identification was based on phenotypic traits, including color, spot
16 pattern and the size and shape of the metatarsal tubercle (Berger, 1977; Günther,
17 1990), but this method is not fully reliable. Therefore, we also drew a small sample of
18 lymph from an incision made into the web between two toes of a hind foot. The lymph
19 was later subjected to albumin electrophoresis which allows unambiguous
20 determination of the species (Tunmer, 1973) and, hence, provided a check of the
21 initial phenotypic assignment. Sex was determined from the presence (male) or
22 absence (female) of vocal sacs and thumb pads. To ensure sexual interest we kept
23 only males ≥ 45 mm, which – without exception – all had swollen thumb pads.
24 Females were only kept when swollen with eggs and/or caught in amplexus without
25 emitting a release call.

26 Between this catching and handling procedure and the start of the choice
27 experiment, all frogs were kept in cages (1.5 x 1 x 0.5 m) for a few hours to several
28 days, separated by sex and species, and individually marked with numbered waist
29 bands (cf. Emlen, 1968). These cages were placed at the shore of another pond in
30 such a way that the animals had access to both, water and land. After having
31 completed its choice experiment, each frog was freed from the waist band and
32 released back into his home pond, but only after marking him with an incision into
33 one foot web to avoid repeated use of the same individual.

34

35

Insert Figure 2 about here

1 **Experimental setup**

2

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

24

25 **Variables and statistics**

26

27 From a car, parked about 1.5m meters off the test arena, we recorded every 30
28 seconds the following variables for all three animals:

29 *Position* - whereabouts of the frog within the grid of the tank bottom

30 *Activity* - defined as a change from one grid cell to another since the last position was
31 recorded

32 *Climbing* - defined as the presence or absence of climbing movements at the wire-
33 screen, which indicate an attempt to closely approach the target animal
34 behind the partition.

1 *Calling* - vocalization of males (yes/no) during a 30-second period.

2 Since calling was extremely rare, it was not further considered in the analyses.
3 For the other three variables, data from all 120 recordings, that constitute an
4 experiment (60 from each of the two 30-minute sessions), were pooled to yield
5 measures for the individuals' total amount of *activity* and the *time* spent in various
6 sectors of their compartments. *Time* was calculated by multiplying the scan interval
7 of 30 seconds by the no of recordings. The test animal's *interest* in the target frogs
8 was measured by comparing the *time* it spent in the two sectors closest to the
9 partition (Figure 2) against a random distribution. *Preference* was expressed as the
10 difference between the *times* spent in the sectors adjacent to the LL- and LR-target
11 frogs, respectively, and by the difference in *climbing* directed towards them. Based
12 on expectations from hybridogenetic reproduction (see Introduction) and from
13 previous empirical results (Abt & Reyer, 1993), the alternative to the null hypothesis
14 of no preference was a preference for LL-individuals. Hence, the region of rejection
15 was only at one end of the sampling distribution, which called for a one-tailed test.
16 Since *R. lessonae* and *R. esculenta* differ in average size and activity (Blankenhorn,
17 1974; Günther, 1990) and these variables are known or suspected to affect mate
18 choice - even within species (e.g. Howard, 1988; Marquez, 1993) - we further tested
19 whether *preference* was related to *activity differences* between the two target animals
20 and to *body size*, i.e. to the difference in the SVL of the two target animals.

21

22

23 **RESULTS**

24

25 We tested a total of 97 frogs, but had to discard data from 62 individuals for the
26 following reasons: (a) the – probably scared - test animal remained motionless for
27 more than 50% of the observation period ($n = 21$); (b) the test animal never changed
28 tank sides to inspect both target frogs at least once ($n = 28$); (c) albumin
29 electrophoresis corrected the initial species assignment based on phenotype and
30 revealed that both target frogs had been of the same species ($n = 10$) or one was a
31 *R. ridibunda* ($n = 1$); (d) by mistake an individual was tested twice ($n = 1$); (e) the test
32 frog escaped from the arena during the experiment ($n = 1$). Criteria (a) and (b) had
33 been defined a priori, whereas (c) – (e) emerged only during the experiments and
34 analyses, respectively. This left us with data from 35 frogs (11 LR- females, 7 LL-

1 females, 7 LR-males and 10 LL-males) which moved between the opposite ends of
2 the test arena and clearly inspected both target animals.

3

4

Insert Figures 3 and 4 about here

5

6 Figure 3 shows that test animals of both species and sexes spent significantly
7 more time in the two compartment sectors bordering the wire partitions than
8 expected by chance. This is true, no matter whether expectation is calculated from
9 the number of sectors (0.28) or from the total length of the central compartment's
10 edges (0.56) which the animals preferred (all $p < 0.001$; range of t-values: |13.03| to
11 |84.19|, range of df: 6 to 10; two-tailed t-tests for pairwise comparisons between
12 observed and expected times). When total time near partitions is broken down by
13 species of the target frogs, it turns out that test animals of both species behaved in
14 the same way (Figure 3): females spent significantly more time with LL- than with LR-
15 males (both $p < 0.05$; *R. esculenta*: $t=-2.442$, $df=10$, *R. lessonae*: $t=-2.073$, $df=6$; one-
16 tailed t-tests for pairwise comparisons between observed and expected times)
17 whereas males showed no preference for either LL- or LR-females (both $p \geq 0.528$;
18 *R. esculenta*: $t=0.669$, $df=6$, *R. lessonae*: $t=0.124$, $df=9$).

19 A more detailed analysis (MANOVA, Table 1) which included the location of
20 the target frogs (left or right compartment) as well as activity and size differences
21 between them confirmed and extended the above result: both the time spent with the
22 target frogs and the frequency of climbing at the partitions is independent of the test
23 animal's species, but differs between the sexes (Figure 4). While males did not
24 discriminate between females of the two species, females spent significantly more
25 time near *R. lessonae* males and climbed more at the partition separating them. The
26 analysis also showed that this female preference for LL-males could not be explained
27 through differences in the target males' activity and/or body size (Table 1). This, and
28 the fact that target animals almost never vocalized, suggests that females can
29 choose LL-males by their phenotype, independent of their behavior and size.

30

31

Insert Table 1 about here

1 DISCUSSION

2

3 Causes for the observed sex differences in mate choice

4

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

13 1) *Costs of mating with the wrong species* - The consequences of mating with a
14 hybrid are the same for both sexes: no genetic contribution to the next generation,
15 either because the offspring are not viable (LR x LR matings) or because they
16 exclude the L-genome when sexually mature (LR x LL and LL x LR matings; cf.
17 Introduction and Figure 1). However, the lifetime fitness cost of such a reproductive
18 failure is likely to be higher in females than in males. Females usually spawn only
19 once per season (Günther, 1990) and, hence, lose the reproductive effort of a whole
20 year, whereas males stay at a pond for several weeks and can mate repeatedly (Abt
21 and Reyer, 1993; Günther, 1990; Schuchardt and Klingel, 1984). Moreover, water
22 frogs have a strongly skewed operational sex ratio (OSR) with males outnumbering
23 females; this is typical for prolonged breeders in anurans (Wells, 1977). As a result,
24 females usually have the – at least theoretical - option of choosing the preferred LL-
25 males, whereas for males unpaired LL-females are often not available. In such a
26 situation, the costs of erroneously amplexing a LR-female may be low, compared to
27 the costs of discriminating between females of the two species and to the benefits
28 from choosing large females, which are discussed in the following paragraph.

29 2) *Benefits from mating with the right size* – For mechanical reasons, optimal
30 fertilization success requires size-assortative mating, i.e. female/male size ratios
31 which are not too extreme (Davies and Halliday, 1977; Ryan, 1985; Gerhardt et al.,
32 1987; Robertson, 1990). Within the suitable size range, however, selection will act on
33 males to prefer large females of higher fecundity (cf. Blankenhorn, 1974, 1977;
34 Notter, 1974) and on females to chose smaller or at most equal-sized males,

1 because this will ease swimming and spawning (Licht, 1976; Robertson, 1986).
2 Since, on average, *R. esculenta* is bigger than *R. lessonae*, size cues alone should
3 direct males towards hybrid females, but females towards parental males. Thus, for
4 females both, benefits from mating with the right size and costs from mating with the
5 (genetically) wrong species, predict the observed preference for LL-males. In males,
6 however, the genetically beneficial choice of LL-females is opposed by a size-related
7 preference for LR-females. This may explain their indiscriminate behavior.

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

27

28 **Mate choice and mating in nature**

29

30 How relevant are the side associations measured in our study for mate choice and
31 mating patterns in nature? In anurans, male vocalization plays a dominating role in
32 attracting females; consequently, studies of female choice almost exclusively use
33 phonotactic approaches to measure it. However, anecdotal observations and
34 experimental evidence suggest that movements towards and away from (even non-

1 calling) males as well as temporal changes in next-neighbor distances also reflect
2 sexual interest and are used to compare among different potential mates
3 (Blankenhorn 1974, 1977; Abt and Reyer, 1993; Rothmair, 1994; Bergen et al. 1997;
4 H.-U. Reyer and G. Frei, unpubl. data). Moreover, the female preference for *R.*
5 *lessonae* over *R. esculenta* males found in our experiment is consistent with results
6 from other studies, indicating that LL-males are more successful in reproduction than
7 LR-males. In an experiment measuring the combined effects of female choice and
8 male-male competition on actual mating combinations, Bergen et al. (1997) found
9 males to be successful in a ratio of 57% LL : 43% LR. This is close to the 60:40%
10 time ratio in favor of LL-males that we found (Fig. 3) and the 66:44% ratio among
11 fertilized egg masses found in a natural pond, where 51% of all clutches originated
12 from the LL x LL mating combination (G. Abt, unpubl. data; see also Blankenhorn,
13 1977; Radwan and Schneider, 1988). In other natural ponds, females were found in
14 amplexus with LL- and LR-males, respectively, even in the ratio of 81:19% (H.-U.
15 Reyer, unpubl. data). These latter ratios, which are already corrected for
16 expectations from random mating, suggest the existence of additional mechanisms
17 which skew the success of LL-males beyond the preference of 60:40% found in this
18 study.

19 Potential candidates for such mechanisms include: (a) approach to
20 (aggregations of) preferred males from some distance by using their mating calls for
21 orientation (Roesli and Reyer, 2000); (b) avoidance of fast movements, direct contact
22 and other cues which normally stimulate the indiscriminate males to forcefully amplex
23 (Bourne, 1992; Emlen, 1976; Grüsser and Butenandt, 1968; Notter, 1974; Robertson,
24 1986; Ryan, 1985; and own observations), (c) vertical body positions, release calls
25 and provoking of fights to get rid of amplexant males (Abt and Reyer, 1993;
26 Blankenhorn, 1977) and (d) „cryptic“ choice through reducing the clutch size when
27 spawning with a LR-male (Reyer et al., 1999). (e) Finally, the better mating success
28 of LL-males may reflect a male trait, rather than a female preference. In this respect,
29 however, the evidence is controversial. Blankenhorn (1974, 1977) suggested that *R.*
30 *lessonae* males gained more mates because of their appropriate sexual, rather than
31 aggressive, behavior in the presence of females, whereas Bergen et al. (1997)
32 concluded that *R. esculenta* males were relatively successful in achieving matings,
33 because they showed high levels of aggression against other males, including

1 competing *R. lessonae*. Ongoing experiments suggest that the competitive ability of
2 males may vary with the LL/LR-ratio (H.-U. Reyer, unpubl. data).

3 Whatever the precise mechanism, the female preference for LL-males found in
4 this and other studies (Abt and Reyer, 1993; Reyer et al., 1999; Roesli and Reyer,
5 2000) is consistent with the fact that in mixed populations of *R. lessonae* and *R.*
6 *esculenta* the relative frequencies of the four possible mating combinations (LL x LL,
7 LL x LR, LR x LL, LR x LR) are shifted from those expected under the assumption of
8 random mating to those involving LL-males. This assortative mating pattern results in
9 a reduced number of LR-offspring, which is crucial for promoting coexistence of the
10 sperm-dependent hybrid and its sexual host (Som et al. 2000, Hellriegel and Reyer,
11 2000). However, further studies are needed to answer the question how mating
12 behavior affects the population dynamics in detail, especially whether and how it also
13 contributes to the markedly different LL/LR-ratios found in natural ponds
14 (Blankenhorn 1974, 1977; Berger, 1977; Hohenweg, 1999). These investigations are
15 presently under way.

16
17

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Table 1

Results from a MANOVA relating the differences (LL-LR) in time spent with target frogs and in climbing at their partitions to species, sex and species * sex interaction of the test animal (factors) and to the side, activity differences and size differences between target frogs (covariates). Shown are *df*-, *F*- and *p*-values for multivariate and univariate tests. Significant results are printed in bold.

dependent variables independent variables	multivariate test			univariate tests				
	<i>df</i>	<i>F</i> (Wilks' λ)	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
species	2, 26	1.111	0.344	1, 27	0.659	0.424	0.197	0.661
sex	2, 26	4.605	0.019	1, 27	9.316	0.005	5.608	0.025
species * sex	2, 26	0.620	0.546	1, 27	1.157	0.292	0.183	0.672
side of target frog	2, 26	0.544	0.587	1, 27	1.022	0.321	0.168	0.685
activity difference	2, 26	1.197	0.318	1, 27	0.045	0.833	1.751	0.197
size difference	2, 26	1.676	0.207	1, 27	0.092	0.764	1.435	0.241

Figure 1 Possible mating combinations and resulting offspring (cells 1-4) in mixed populations of *R. lessonae* (genotype LL) and *R. esculenta* (genotype LR). ✕ 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 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_yL_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 † in cell 4 indicates that these tadpoles don't survive to metamorphosis.

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 x 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.

Figure 3 Proportion of time which the test frogs spent with either target frog (white bars) and with *R. lessonae* (grey bars). Total proportion (white) is expressed in relation to the total observation time of 60 minutes, the proportion spent with *R. lessonae* (grey) 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, i.e. a random distribution between LL- and LR-target individuals.

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

Figure 1

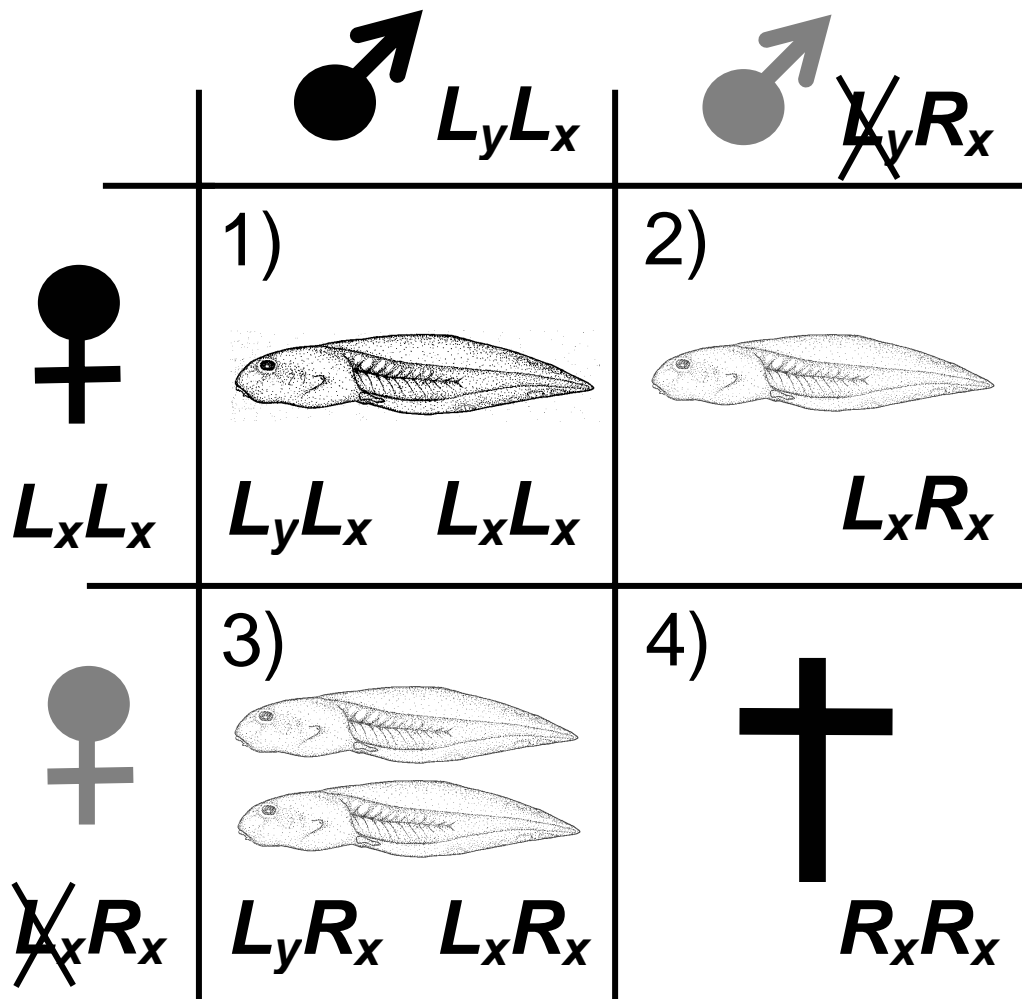


Figure 2

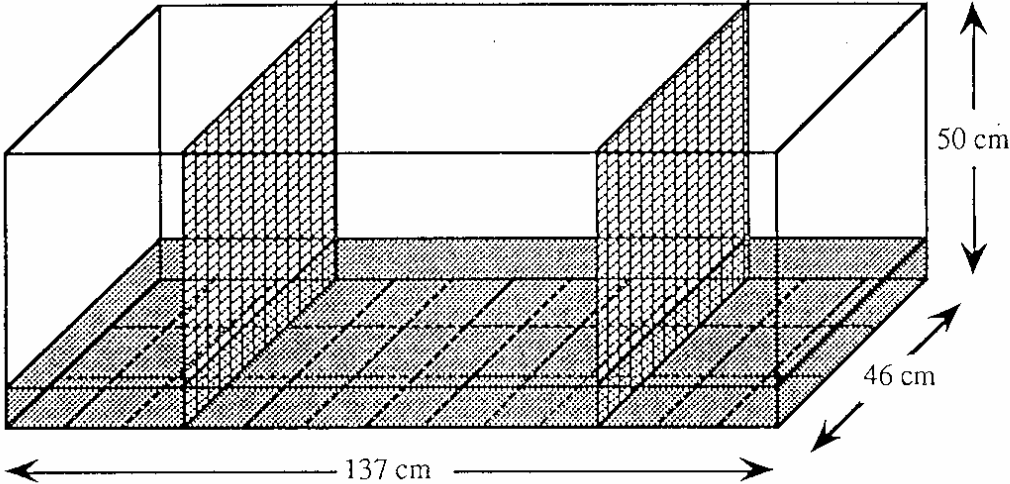


Figure 3

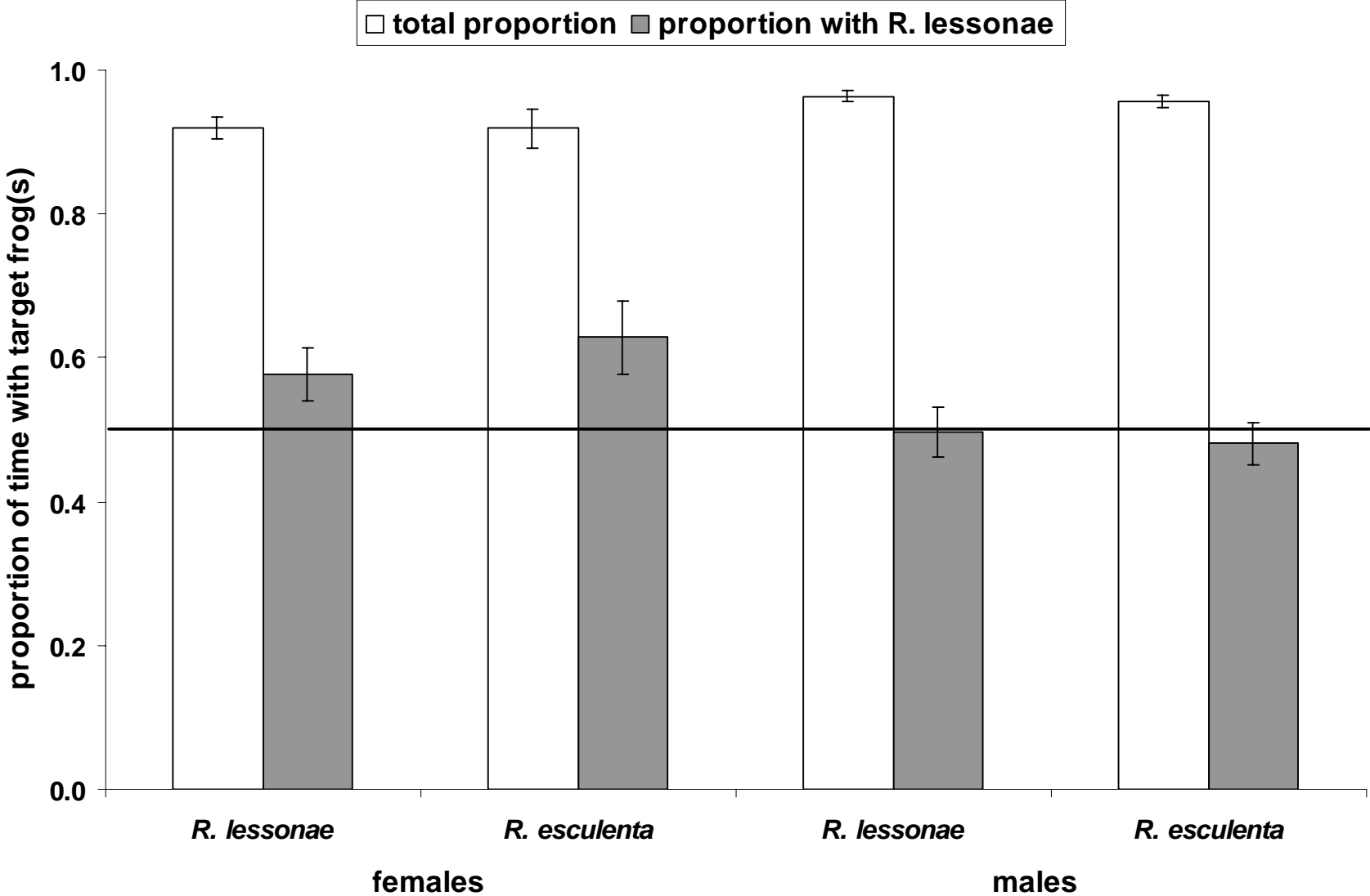


Figure 4

