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Hybrid Female Matings Are Directly Related to the Availability of *Rana lessonae* and *Rana esculenta* Males in Experimental Populations

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In the hybridogenetic frog complex *Rana lessonae* and *Rana esculenta*, mate choice based on genetic benefit could be an important form of sexual selection. The hybrid *R. esculenta*, must coexist with the parental species *R. lessonae*, which functions as a genetic host. The two types occur in varying ratios in natural populations depending on environmental conditions of the aquatic habitat. In highly disturbed habitats such as gravel pits, *R. esculenta* is most common, whereas *R. lessonae* is more frequent under less disturbed environmental conditions. To examine the influence of different proportions of males of the two types on mating success, we subjected hybrid females to different ratios of *R. lessonae* and *R. esculenta* males in artificial pools. Our results show that females mated according to the availability of the two types of males in the pools. However, combining data over all ratio treatments suggested a slightly higher mating frequency for *R. lessonae* males than for *R. esculenta* males, although the *R. esculenta* males behaved more aggressively, called more often, and tended to orient more frequently toward females than did *R. lessonae* males. This, plus results from previous mate choice studies, suggests that *R. esculenta* females have a preference for mating with *R. lessonae* males because of genetic benefits; but which mate a female finally mates with is also influenced by other processes, such as the availability of the two male types at the time of mate choice as well as the strength of male-male competition.

SINCE Darwin stated that most animals do not mate randomly, biologists have sought mechanisms for the basis of sexual selection. Competition within the same sex and mate choice between the sexes have been identified as the main processes (Trivers, 1972), and they play an important role in anuran mating systems (Wells, 1977a; Arak, 1983; Sullivan, 1989). In some anurans, male-male competition is the primary mechanism of sexual selection (Davies and Halliday, 1979; Wells, 1977b), whereas in others it is primarily female choice (Whitney and Krebs, 1975; Ryan, 1985; Arak, 1988). Which of the two mechanism dominates the mating system of a species depends on duration of the breeding season (Wells, 1977a).

In anurans, there are two patterns that represent ends of a continuum, explosive breeding and prolonged breeding (Wells, 1977a). In an explosive breeding system, males and females arrive synchronously at the breeding site, and both sexes stay only for a few days or weeks. In most of the cases, males compete directly for females (i.e., scramble competition). Although larger males can displace smaller males on females, and therefore often have a higher mating success, variance in male reproductive success is limited by the short time available for breeding. In a prolonged breeding system, males remain at ponds for several weeks or months, and females arrive asynchronously over the whole breeding period. This results in strongly male-biased operational sex ratios. Under these conditions, superior males have more opportunity to mate with more than one female (leading to higher reproductive variance); females have more time to choose among males and selectively mate with those possessing certain traits or resources, including call frequency or call rate (Ryan, 1985; Klump and Gerhardt, 1987; Arak, 1988), body size (Morris, 1989; Morris and Yoon, 1989; Robertson, 1990), and territory quality (Cherry, 1993).

Some of these male traits are known to provide females with direct benefits for reproductive success, for instance, egg deposition in sites with low predation risk (Wells, 1977b; Howard, 1978), or high fertilization rate (Robertson, 1990). However, it has not been demonstrated yet that females choose males that possess good genes and transmit an indirect advantage to their offspring in terms of growth, survival, or reproductive success (see review in Howard et al., 1994)

European waterfrogs of the hybrid complex *Rana lessonae* and *Rana esculenta* are a suitable system to study mate choice due to indirect genetic benefits. In this prolonged breeding system, males establish choruses at potential breeding sites during late spring and summer and do not defend territories or provide parental care. Females move asynchronously to these sites for mating, and, because of a special reproductive mode, there is strong selection for choosing the genetically correct mate (Abt and Reyer, 1993; Engeler, 1994). The hybrid *R. esculenta* (E-phenotype, LR-genotype) originated from matings between *R. lessonae* (L-phenotype, LL-genotype) and *R. ridibunda* (R-phenotype, RR-genotype). Both parental species occur in many places in Europe, but in Switzerland only one parental species, *R. lessonae*, exists (Blankenhorn, 1974; Günther, 1990). The hybrid has a special form of reproduction known as hybridogenesis (Berger, 1977; Keegan-Rogers and Schultz, 1984; Graf and Polls Pelaz, 1989). During gametogenesis, the hybrid excludes the *R. lessonae* (L) genome and only transmits a clonally duplicated *R. ridibunda* (R) genome to its eggs or sperm. Therefore, to produce viable offspring, the hybrid must regain the lost L-genome by mating with *R. lessonae* each generation. As a consequence, the hybrid cannot exist alone but must coexist with one of the two parental species. In Switzerland and at our study populations, it is syntopic only with *R. lessonae*.

In such mixed populations, matings occur between *R. lessonae* adults, producing *R. lessonae* offspring, and between the hybrid and *R. lessonae*, resulting in viable *R. esculenta* progeny. Matings between hybrids occur but in most cases do not result in viable offspring. A number of lethal factors come together on the clonally inherited R-chromosomes and lead to death of the homozygous progeny during the larval stage. This has been observed both in nature and in laboratory experiments (Berger, 1977; Uzzel et al., 1980; Semlitsch and Reyer, 1992). Consequently, there must be strong selection for hybrid individuals to live in mixed populations and find *R. lessonae* mates, but there is a disadvantage for the parental species to function as a genetic host.

In addition to this conflict of interests, the probability to mate with the genetically correct partner is further confounded by the availability of potential mates. *Rana lessonae* and *R. esculenta* occur in different ratios among various types of ponds (5–95% of either species). In disturbed environmental conditions (e.g., gravel pits), the hybrid is relatively more abundant than the parental species, whereas *R. lessonae* dominates in woodland ponds or natural bogs. The purpose of our research, therefore, was to determine whether different ratios of *R. lessonae* and *R. esculenta* males in a pond influence mating frequencies. We also tested the effects of body size and various behavior patterns of the two male types to elucidate the processes leading to successful mating.

MATERIALS AND METHODS

We measured the effect of different proportions of *R. esculenta* (E-phenotype, RL-genotype) males and *R. lessonae* (L-phenotype, LL-genotype) males on the mating success of *R. esculenta* females. To achieve this, we subjected *R. esculenta* females to three different experimental ratios of hybrid and parental males in artificial ponds: 3 RL:7 LL, 5 RL:5 LL, 7 RL:3 LL. The three treatment ratios were replicated unequally because of the short breeding season in 1993 and the low availability of gravid females.

Experimental ponds.—We used six plastic wading pools (2.5 m in diameter, 0.43 m deep) as experimental ponds. The pools were positioned close together in two parallel lines in a fenced field at the University of Zürich-Irchel. Each pond was surrounded by a 1 m high wire-mesh fence of which the upper 10 cm was bent toward the inside of the pool to prevent frogs from jumping out or climbing over. The pools were then filled with tapwater to a level of 15 cm. In the center of each pool, we placed a wire cage (60 cm long, 20 cm wide, 60 cm high) between two bricks. Attached to each brick, we fastened a floating piece of wood (25 cm long, 20 cm wide, 2 cm thick) on a short string. Both the bricks and wood served as perches and shelter for the frogs. The wire cages were used for acclimating females to the new environment before the start of each test. Other than these necessities, we left the ponds simple so that behavioral observations were not obstructed. Previous mate choice studies under even more impoverished environmental condititions (Abt and Reyer, 1993; Engeler, 1994) and the frequent observation that frogs will even amplex while being carried in buckets had shown that such artificial conditions do not impair the normal mating behavior.

Experimental animals.—For the experiment, we collected 279 waterfrogs between 13 May and 4 July 1993 from two populations: a small pond near the Katzensee, Kanton Zürich, consisting of 42% *R. lessonae* and 58% *R. esculenta*, and a larger pond near Gütighausen, Kanton Schaffhausen consisting of 52% *R. lessonae* and 48% *R. esculenta* (RDS, unpubl. data). In all, we used 56 frogs from the Katzensee population and 223 frogs from the Gütighausen population; however, frogs were never mixed.

Frogs were hand-collected at night once per week and brought into the laboratory for processing. We measured body size (snout–vent length in millimeters), permanently marked individuals by toe clipping, and then identified the species by performing electrophoresis on lymph albumin or on LDH from toe tissues (Tunner, 1973, 1979; Vogel and Chen, 1977). Frogs were also given a temporary waistband (color-coded plastic 10 mm × 10 mm attached around the waist with dental floss) to distinguish their sex and species during observations. After processing, all animals were placed into outdoor cages and fed crickets, usually within 12 h of capture. During the nontesting period, the frogs were held in one of three outdoor aquatic cages with screen-covered sides and top. The bottom of each cage consisted of a plastic tub (1 m long, 1 m wide, 30 cm deep) filled with 20 cm of water and some floating pieces of wood for perches. The water was changed once a week, and the frogs were fed crickets (12–15 mm in length) daily ad libitum.

Experimental protocol.—The experiment was performed from 14 May to 7 July 1993. The different ratio treatments as well as the males and females within treatments were randomly assigned to the six pools. The evening before a test, 10 males were selected from the holding cages and randomly assigned to pools according to the ratio treatment. They were acclimated overnight, and then the next morning between 0830 and 0900 h, a female was secured in the wire cage for 2 h. We then released the female by remotely opening both sides of the cage to allow her and the males to move freely into and out of the cage. During the tests, a tape recording of a natural mixed chorus of *R. lessonae* and *R. esculenta* was played at 40-min intervals, i.e., a 30-min chorus, followed by 10 min of silence.

We observed frogs in the six pools for 3–5 h per day depending on how quickly they mated. During that time, each pool was observed for 10 min once per hour and the following 10 behavior patterns were recorded every minute: hiding, sitting, clinging to the cage, floating on the water's surface, swimming, amplexing (all for both sexes), calling, fighting, jumping at the female (only for males), and fleeing from the male (only for females). Aided by a grid drawn on the bottom of the pools, we also noted every minute the position of all 10 males relative to the female as close (< 10 cm apart), moderate (10–50 cm apart), and far (> 50 cm apart). A test lasted until a female achieved amplexus or for a maximum of three days. Detailed behavioral data were only taken on the first day of a test but included at least two 10-min observation periods for each pool.

When a pair was observed in amplexus, we waited until the female had laid a clutch of eggs to ensure the pair would not separate prematurely. Thereafter, we caught the pair, separated the male from female, and noted the species and time to amplexus. Both were then returned to their source pond. The other males were placed back into the holding cage, the pool was drained, cleaned, refilled with fresh tapwater, and again set up with 10 randomly assigned males from one of the three holding cages. This removal of once successful males and reshuffling of the others was chosen to guarantee that we tested for the effects of genotype ratios rather than of dominant individuals. Females that did not pair in the first test were tested again under the same conditions 2–3 days later. Then, if they did not mate, they were released to their source pond; also, males that showed no interest in females for several days were released, because this suggested that they were shy of the experimental environment.

It was impossible to use only "naturally ready" females that were field-collected in amplexus for all tests; therefore, we injected all females with luteinizing-releasing hormone (LRH; H-7525, Bachem, Inc.) to stimulate them to similar levels of breeding readiness. LRH initiates a cascadelike effect, which finally leads to an output of oestradiol within natural physiological limits (Becker et al., 1992). This results in normal ovulation and spawning behavior, whereas direct injections of oestradiol may change the usual behavior because of a sudden stimulation of ovulation in an unbalanced hormonal environment. The evening before testing, females were injected with LRH (25 μg per 100 g body weight) and kept isolated in plastic containers with a small amount of water until being placed into the acclimation cage the next morning. This procedure has been successfully used on hundreds of females for purposes of artificial fertilization in this species and other anurans (e.g., Smith-Gill and Berven, 1980; Semlitsch, 1993a, 1993b).

Altogether we performed 30 experiments, 10 replicates each for the 3:7, 5:5, and 7:3 L/E-male ratios. Successful matings occurred in only 21 of the 30 experiments. In seven of these 21 cases, we did not observe amplexus directly, because it took place during the night or early on the morning of the second day. However, in all 21 cases, we were able to determine the species of male that successfully mated by allowing the fertilized clutch of eggs to hatch and typing the tadpoles using electrophoresis (LDH; Vogel and Chen, 1976, 1977). Because females were all known to be *R. esculenta*, the pattern of LDH alleles for tadpoles correctly identified the sire as *R. esculenta* or *R. lessonae*.

Statistical analyses.—All activity responses we observed were converted to percentages of time and were angularly transformed by arcsine square-root. All time and size measurements were log-transformed before analysis. The specific statistical tests we used are mentioned with the respective results. All tests were performed with STATGRAPHICS (STSC, Inc.), and a significance level of $P < 0.05$ was used throughout. Power of tests was calculated according to Cohen (1988).

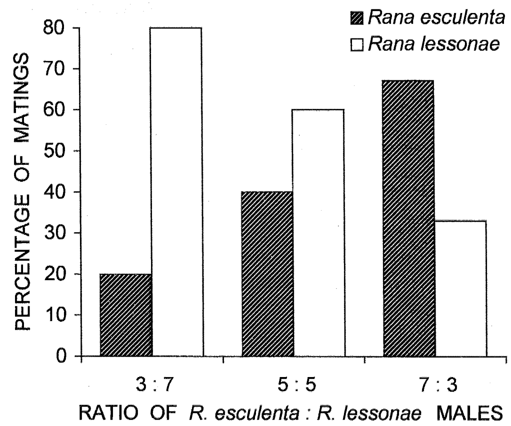

Fig. 1. Percentage of successful matings of *Rana esculenta* (hatched) and *Rana lessonae* (open) males in the three ratio treatments of males (3 *R. esculenta*:7 *R. lessonae*, 5 *R. esculenta*:5 *R. lessonae*, 7 *R. esculenta*:3 *R. lessonae*). A total of 21 successful matings were observed in the experimental ponds.

RESULTS

We used equally sized *R. esculenta* females for all experiments to eliminate the confounding effect of female size differences. Two of the 21 spawning females escaped before we had measured them. Among the other 19, SVL varied from 71–75 mm but did not differ significantly among the male ratios (ANOVA, F = 0.41, df = 2,16, $P = 0.671$). Therefore, we can exclude female size as being important in the subsequent results.

At the start of each test, females had been under the hormone influence for different periods of time, depending on weather conditions (e.g., rain, cool temperatures, or cloudy weather). To test whether these differences in "injection time" affected the time-lapse from the start of the experiment to amplexus ("mating time"), we performed two Spearman rank correlations. The first correlation was for females with short mating times (20–350 min), the second for those taking longer (800–1700 min). In both data sets, there was no correlation between the injection time and the mating time (Spearman correlation coeffcient; $r_s = -0.105$ and $r_s = -0.012$, respectively). Therefore, possible differences that may have occurred in the time until amplexus are probably not a result of the different injection times.

To determine whether the probability of achieving amplexus depended on male type or simply on the availability of males in the pond, we tested the mating success for the two species of males with three different male ratios in the pools: 3 *R. esculenta*:7 *R. lessonae*, 5 *R. esculenta*:5 *R. lessonae*, 7 *R. esculenta*:3 *R. lessonae* (Fig. 1). The mating success of neither *R. esculenta* nor *R. lessonae* was significantly different from the expected frequency of males (contingency log-likelihood G-test: G = 2.58, df = 2, $P > 0.25$). Thus, males of both species were mated according to the availability of each species in the experimental pools. Even when results from all three treatments were pooled, there was no significant difference in the mating frequency of *R. esculenta* (43%) and *R. lessonae* (57%) males from the expected ($\chi^2 = 0.43$, df = 1, $P > 0.5$), albeit slightly biased toward *R. lessonae*. Admittedly, the statistical power of our test is weak. With a sample size of 21 successful matings and a deviation of only 7% from an equal distribution, the one-tailed probability for rejecting the null hypothesis with $P \leq 0.05$ is only about 0.20. However, the observed 43:57% mating ratio in these experiments with male-male interaction differs significantly from the 9:91% preference ratio found in previous experiments without male-male interaction ($\chi^2 = 4.78$, df = 1, $P = 0.029$; power = 0.70). When 22 *R. esculenta* females were given a choice between one *R. esculenta* and one *R. lessonae* male confined to opposite sides of an aquarium, 20 of them spent more time on the side of the *R. lessonae* male (Abt and Reyer, 1993; Engeler, 1994). In combination, these results show that female preference for males of the parental species in simple choice experiments is superimposed and confounded by male-male competition in more realistic mating situations.

The time until amplexus varied from 20–1700 min but did not differ among the ratios, neither when the species were considered separately (*R. esculenta* males: ANOVA, F = 2.03, df = 2,6, $P = 0.212$; *R. lessonae* males: F = 0.47, df = 2,9, $P = 0.639$) nor when data from both species were pooled (F = 0.61, df = 1,19, $P = 0.446$).

To test for the effects of male body size on achieving amplexus, we compared (within species) the SVL of successful males with those of the unsuccessful males for the corresponding experiment. For the ratio 3 *R. esculenta*:7 *R. lessonae* males, a mating with *R. esculenta* males was missed, and therefore, we could not distinguish between the successful and unsuccessful *R. esculenta* males. Student's *t*-tests were not significant for either species of male (for *R. esculenta*: 5 E:5 L, $t = 0.97$, df = 4, $P = 0.389$; 7 E:3 L, $t = 0.03$, df = 5, $P > 0.5$.; for *R. lessonae*: 3 E:7 L, $t = 0.33$, df = 5, $P > 0.5$; 5 E:5 L, $t = 0.03$, df = 8, $P > 0.5$; 7 E:3 L, $t = 0.09$, df = 1, $P > 0.5$). To determine whether size-assortative mating occurred, we used a Spearman rank correlation between sizes of amplexed males and females but found no relationship (*R. lessonae* males $r_s = 0.18$; *R. esculenta* males $r_s = 0.0$). Except for one female, all females mated with smaller males.

Potential effects of male behavior on mating success were tested by using discriminant analyses with a nested design. The behavior of males was compared: (1) between situations of successful and unsuccessful mating; (2) among the three different male ratios; and (3) between the two species. Overall, males oriented more toward females, fought more often, and had a higher calling rate in pools where frogs successfully mated than in pools where they were unsuccessful (Wilks' lambda = 4.81, df = 4,32, $P = 0.004$; Fig. 2). These differences were mainly the result of a significant species effect (Wilks' lambda = 2.41, df = 8,64, $P = 0.02$). Although the two species had similar values for sexual and aggressive activities in pools where no mating occurred, *R. esculenta* exhibited considerably more orientation toward females, fighting with males, and more calling than *R. lessonae* in pools with mating (Fig. 2). There was also a species effect on swimming activity which was lower in pools with successful mating than in pools with unsuccessful mating for *R. lessonae* but not for *R. esculenta*.

The effect of male ratio treatments on the overall behavior of frogs was not significant for either situations of successful mating (Wilks' lambda = 0.43, df = 8,12, $P = 0.88$) or unsuccessful mating (Wilks' lambda = 1.64, df = 8,38, $P = 0.15$). In addition, there was no species effect within the male ratio treatments for situations of successful mating (Wilks' lambda = 1.78, df = 4,6, $P = 0.25$) or unsuccessful mating (Wilks' lambda = 0.91, df = 4,19, $P = 0.48$).

DISCUSSION

Schultz (1969) stated that the origin of hybrid systems is only possible through an imperfect mate selection system. A *R. esculenta* female mating with the wrong type (i.e., a *R. esculenta* male) has strong negative effects on her reproductive success because of inviable offspring. Thus, females should be under strong selection to avoid mating with hybrid males. Yet, matings occurred according to the frequencies of male types in the experimental pools. The slightly higher proportion of *R. lessonae* than *R. esculenta* matings (57% and 43%) was not significant, and no difference in the time to amplexus between the two species of males was found. These results, together with previous experiments from our laboratory, suggest that two different mechanisms of sexual selection are likely interacting to produce the observed mating frequencies: female choice and male-male competition.

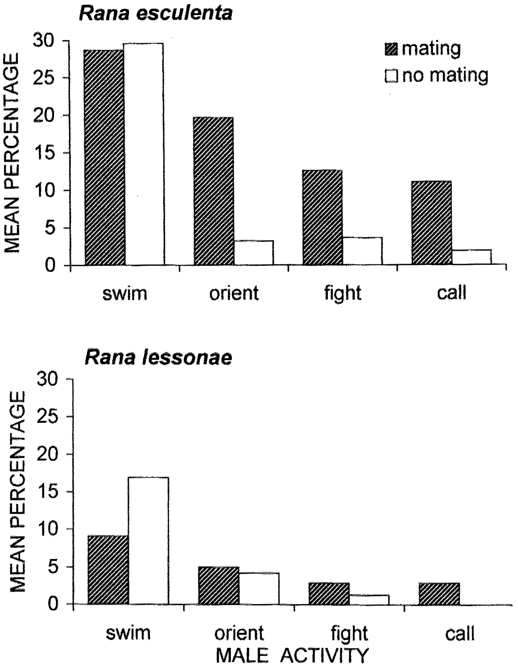

Fig. 2. Percentage of time spent by *Rana esculenta* and *Rana lessonae* males in swimming (swim), orienting toward females (orient), fighting with other males (fight), and calling (call). Bars show the mean for experiments where successful mating (hatched) and no mating (open) occurred with *R. esculenta* females.

Abt and Reyer (1993) and Engeler (1994) both showed that female *R. esculenta* and *R. lessonae* in a large aquarium, subjected simultaneously to two noninteracting males (one of each type), preferred *R. lessonae* males as mates. Males subjected to the same choice experiments showed no preference for either of the two female types (Engeler, 1994). This sexual difference in mate preferences is to be expected because females lose much more from a wrong mating than do males (Trivers, 1972; Abt and Reyer, 1993). In the above experiments, females probably used visual and chemical cues to distinguish between the two male types because of the absence of calling, but calls also seem to work equally well (Engeler, 1994). Such cues are likely to be easy to analyze for females in simple two-choice experiments. But as soon as the choice situation becomes more complex, the females can be expected to be more easily confused and to make incorrect decisions (i.e., matings that lower offspring success; Gerhardt et al., 1994)). In our experiments, females did find such a complex situation, i.e., several males of each type per experiment. In pools with an excess of males of the parental species, correct males were easier to detect for the females than in pools with few males of the parental species, hence more "mistakes" in choosing the correct male as the E:L-male ratio increases.

In many aurans, male-male competition is the primary force of sexual selection (Howard, 1978; Davies and Halliday, 1979; Arak, 1983). Two different mechanisms of male-male competition could also play an important role for the waterfrog mating system, the spatial organization of male choruses at the pond and behavioral differences between *R. lessonae* and *R. esculenta* males. The congregations of displaying males in ponds resemble the leks of other vertebrates which females visit solely for the purpose of mating (Blankenhorn 1977; Wells 1977a). Blankenhorn (1977) indicated that most *R. lessonae* males were positioned at the edge of the choruses, with most *R. esculenta* males in the center of the arena. Thus, *R. lessonae* males were assumed to be more successful because they were in the correct position in the arena, i.e., the area which approaching females enter first. Similar position-related mating advantages have been reported for some other lekking species like Sage Grouse, African fruit-eating bats, and bullfrogs (Wiley, 1973; Bradbury 1977; Emlen 1976). In our experimental pools, however, males of both species seemed to be randomly distributed and females were released from the center. Therefore, *R. lessonae* males could have lost the position advantage observed by Blankenhorn.

Blankenhorn (1977) also suggested that *R. lessonae* males were more successful in gaining mates because they reacted with the appropriate mating behavior in the presence of a female, whereas the *R. esculenta* males behaved very aggressively toward both males and females. In our pools, too, *R. esculenta* males showed more aggression than *R. lessonae* males, at least in ponds where mating occurred, but this resulted mainly in fights between males. These fights, together with the more frequent orientation toward females and the higher calling activity of *R. esculenta* may have contributed to the unexpectedly high mating success of hybrid males in our experiments. We have no explanation for the equally surprising low levels of orientation toward females, fighting, and calling of *R. lessonae* males. The fact that their swimming activity was lower in pools with successful mating than unsuccessful mating may indicate that in our pools their overall activity was suppressed. This could have resulted from higher sensitivity of *R. lessonae* males to conditions of captivity, including the high activity of the larger *R. esculenta* males from which the *R. lessonae* males could not escape as they could under natural conditions.

Four studies have previously tried to explain the nonrandom mating pattern in the mating system of *R. lessonae* and *R. esculenta*. Blankenhorn (1974) and Notter (1974) concentrated on the spatial distribution of males and interactions between males, whereas Abt and Reyer (1993) and Engeler (1994) focused on female choice but did not allow males to interact. Both approaches consider only one side of the issue. With our experiments, we tried to simulate more realistic conditions where males had the opportunity to interact and compete, and females were subjected to 10 males and could express a choice. The observed mating frequencies, together with our behavioral observations, suggest that both male-male competition and female choice may work simultaneously to influence mating success. Direct interactions between males and higher aggressive and sexual activity in *R. esculenta* males than in *R. lessonae* males is most likely to be responsible for hybrid males achieving 43% of the matings (nine of 21), in spite of the hybrid females' preference for *R. lessonae* males found in experiments where male-male interactions were not possible (Abt and Reyer, 1993; Engeler, 1994). Female choice is most likely to be responsible for the fact that, in spite of their lower sexual and aggressive activity, *R. lessonae* males achieved as many, if not more, matings (57%) than *R. esculenta*.

Female choice may be expressed in several ways. The first is to avoid amplexus by adopting an almost vertical position in the water (Emlen, 1976; Abt and Reyer, 1993). Another possibility is that a female, paired with an "undesired" male, may swim to a group of males and provoke them to fight with the male on her back. Three times we observed an amplexed female to behave in this manner. In two instances, the amplecting male was displaced. Similar observations have been reported by Blankenhorn

(1974, 1977) and Abt and Reyer (1993) under natural conditions. Females could also exercise postamplexus control and mate choice by ovipositing only a fraction of their clutch with the undesired male. Up to now, it was believed that females laid all their eggs at once (Günther, 1990). However, in our experimental pools, we observed two females that stopped mating after laying some eggs with one male. It could be that these females were not "convinced" by the chosen males (one *R. esculenta* and one *R. lessonae* male) but could not get rid of them until they laid some of their eggs. Such partitioning of clutches may increase a female's chance to remate with a better male within the same season.

Our finding that the mating frequency of hybrid *R. esculenta* females with male *R. lessonae* and *R. esculenta* is jointly influenced by female choice and by the frequency of the two male types has implications for the maintenance of hybridogenetic complexes in L-E and similar systems. In the hybrid fish system of *Poeciliopsis,* for instance, unisexual (all-female) hybrids require sperm from males of the bisexual congeneric parental species for reproduction (McKay, 1971; Vrijenhoek et al., 1977; Schultz, 1982). At high population densities, subordinate males are reported to be forced into suboptimal environments where they have fewer conspecific females and might accept unisexuals as mates (McKay, 1971; Vrijenhoek, 1989). But in populations with only a few males, all males can find optimal habitat, mate with their own females, and would therefore reduce the reproductive success of the all-female unisexual fish. This leads to a system whereby hybrids and parentals would alternatively be favored at different densities and both could coexist indefinitely (Slobodkin, 1961).

In the L-E complex, all ecological conditions leading to high proportions of *R. lessonae* (i.e., climax aquatic habitats with high food resources) will increase the mating frequency between *R. esculenta* females and *R. lessonae* males. These matings result in *R. esculenta* offspring; thus, the relative numbers of *R. lessonae* will decrease. Conversely, conditions favoring high proportions of *R. esculenta* (i.e., gravel pits and disturbed habitats with low productivity) will lead to more matings between male and female *R. esculenta.* Because offspring of these hybrid matings usually do not survive, the proportion of *R. lessonae* will increase. Thus, the frequency-dependent mating success can be expected to have a stabilizing effect on the structure and dynamics of mixed *R. lessonae-R. esculenta* populations.

There are, however, two complications that have to be considered. First, if the same frequency-dependent mating pattern should hold for *R. lessonae* females, then habitats with high proportions of *R. lessonae* will also lead to more matings between male and female *R. lessonae.* This will result in more *R. lessonae* offspring and counteract the increase in *R. esculenta* offspring from hybrid matings. Similarly, habitats with high proportions of *R. esculenta* would theoretically result in more matings between *R. lessonae* females and *R. esculenta* males yielding *R. esculenta* offspring, but, for body size-related reasons, these matings are unlikely to occur. Second, an increase in *R. lessonae* will affect females and males equally because the sex ratio is 1:1, but an increase in *R. esculenta* will result in more females than males (Berger, 1983). Thus, the relationship between mating frequencies and the dynamics of mixed populations will not only depend on the relative roles of female choice and male-male competition under different densities and proportions of *R. lessonae* and *R. esculenta* but also on the sex ratios in the two species. Resolution of this problem is presently being investigated both through long-term population studies and theoretical models.

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