

Social environment and reproductive interference affect reproductive success in the frog *Rana latastei*

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Variation in the social environment produces selection on morphological and behavioral traits. It is less clear how the social environment generates variation in demography through behavioral mechanisms. Theory suggests that one aspect of social environment, relative abundance of heterospecifics, influences the intensity of reproductive interference and its demographic effects. These effects are countered by species recognition and female preferences. We studied the effects of social environment on reproductive success in replicated, mixed breeding populations of two ranid frogs, *Rana latastei* and *Rana dalmatina*, the former being of international conservation concern. We manipulated the social environment of female *R. latastei* experimentally by varying the relative abundance of potential conspecific and heterospecific sexual partners. We measured amplexus frequency and recorded the reproductive success of *R. latastei* females. When conspecific males were relatively uncommon, (1) the absolute and relative frequencies of conspecific amplexus decreased, indicating a breakdown of sexual isolation, (2) oviposition was less frequent, and (3) the percentage of viable embryos in deposited clutches decreased. *R. latastei* females in an environment of low relative conspecific abundance (1:5, *R. Latastei*:*R. dalmatina*) demonstrated 6.8% the reproductive success of females in an environment exclusively with conspecifics. We present a model for the dependence of conspecific amplexus on the social environment. We discuss several mechanisms that may influence reproduction by *R. latastei*, and we support conservation of the species' preferred habitat to reduce opportunities for reproductive interference that occur at shared breeding sites. **Key words:** amplexus, endangered species, frogs, interference, *Rana dalmatina*, *Rana latastei*, red list, reproductive success, sexual isolation, social environment. [*Behav Ecol* 14:294–300 (2003)]

Relationships between the environment and intraspecific variation in behavior offer an opportunity to understand how behavior interacts with external factors to generate population dynamics and maintain variation in nature. Variation in the distribution and abundance of food resources, potential mates, nesting sites, predators, and competitors lead different behavioral strategies to maximize reproductive success (Fryxell and Lundberg, 1998; Lott, 1984; Sutherland, 1996). Social environment is an additional influence on the strategies conferring maximal reproductive success. Variation in social environment arises when groups of interacting conspecifics differ in the proportion of particular morphological characteristics or behavioral propensities. For example, size distribution influences mating tactics of small males in a poeciliid fish and levels of aggressive behavior in salamanders (Brunkow and Collins, 1998; Kolluru and Joyner, 1997). Similarly, the relative frequency of color morphs influences female preferences in a desert lizard (Alonzo and Sinervo, 2001). Further, the frequency of reproductive females influences both male advertisement behavior (e.g., calling rate in mole crickets; Hill, 1998) and female reproductive strategies (e.g., offspring sex ratio adjustment in voles; Aars et al., 1995). Thus, intraspecific variation in social environment can be important for understanding variation in reproductive behavior and the allocation of reproductive effort.

Closely related species can have similar habitat requirements for reproduction, and heterospecific individuals may contribute to social environment. Social interactions with

heterospecifics can interfere with reproduction when low-quality hybrid offspring are produced (Kruuk et al., 1999; Parris, 1999, 2001) or when reproductive success or costs are affected. For example, interspecific agonistic interactions select for dull-colored male flycatchers that experience less interference from more aggressive, sympatric congeners (Alatalo et al., 1994). Further, closely related congeners interfere with reproduction in spider mites and produce reproductive costs for male dragonflies, and reproductive interference from ecologically similar species has been implicated in the replacement of native crayfish by exotics (Schultz and Switzer, 2001; Singer, 1990; Soderback, 1995; Takafuji et al., 1997). Reproductive interference, however, appears to have no influence on population densities of some reptile ticks (Bull and Burzacott, 1994) and appears to play no role in the replacement of red squirrels by invasive gray squirrels in Europe (Wauters and Gurnell, 1999). Thus, although reproductive interference explains some patterns of behavior and morphological character variation, its importance in generating variation in population size and the relative abundance of species is unclear.

Theoretical studies on reproductive interference are also equivocal. One study predicts that reproductive interference leads to unstable coexistence and that competitive exclusion through reproductive interference depends on the initial relative abundance of species (Kuno, 1992). However, other theory suggests that mate choice behavior can reduce interference in some systems, leading to the stable coexistence of species (Som et al., 2000). Further empirical study would help clarify the role of reproductive interference as a mechanism by which variation in social environment impacts populations and communities. Here we present an experimental investigation of the role of reproductive interference in generating variation in demography by examining the relationship among social environment, reproductive

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interference, and female reproductive success in an anuran amphibian.

Amphibians are an excellent group for studying the relationship among social environment, interference behavior, and population vital rates and demography. Many amphibians breed in temporary and permanent ponds, spatially concentrating reproductive activity in aquatic habitat (Duellman and Trueb, 1986). Variation in conspecific and heterospecific components of social environment produce both convergent and divergent evolution on the characteristics of male vocalization (Castellano and Giacoma, 1998; Gergus et al., 1997; Leary, 2001). Over shorter time scales, male treefrogs alter their calling behavior in mixed-species choruses to reduce acoustical interference from heterospecifics and improve mating success (Schwartz, 1987; Schwartz and Wells, 1983, 1984). Additionally, selection can produce high levels of male-male competition (Arak, 1983, 1988), which may sometimes overwhelm female choice (Reyer et al., 1999). Alternatively, the presence of heterospecific males may be “distracting,” decreasing conspecific mating in the absence of observable behavioral interaction (Verrell, 1994). Further, mechanisms of species recognition and sexual isolation in amphibians may depend on the social environment within a few meters of females (Wells, 1977) and on opportunities for comparing conspecifics with heterospecific males (Michalak and Rafinski, 1999), potentially resulting in decreased conspecific mating in sites of syntopy (Bergen et al., 1997; Verrell, 1994).

Studies on amphibians and other systems show that behavioral variation can be generated by the mixed-species social environment and suggest that social environment influences both the cost associated with reproduction and reproductive success itself. Yet despite understanding of the relationship between social environment and variation in the relative reproductive success of individuals, the influence of environmentally induced behavioral variation on the dynamics and demography of populations is largely undeveloped and experimentally unverified (Anholt, 1997), despite earlier claims to the contrary (e.g., Partridge and Green, 1985).

We identified a pair of sympatric, congeneric frogs for which natural history observations suggest the existence of both reproductive interference and reproductive failure at the population level. We focused on the Italian agile frog (*Rana latastei* Bologna) and a common, morphologically similar congener, the agile frog (*Rana dalmatina* Bonaparte). We developed understanding of relationships among environment, behavior, and population processes by examining how variation in social environment affects levels of reproductive interference and the reproductive success of *R. latastei*. We manipulated social environment by altering the relative abundance of reproductive males of the study species. We produced data to reflect on the prediction that opportunities for coexistence depend on social environment (Kuno, 1992) by examining the responses of interference and reproductive success.

In the absence of interspecific interaction, one expects the pairing behavior of female *R. latastei* to be independent of the relative abundance of conspecifics and heterospecifics. Alternatively, the presence of heterospecific potential sexual partners may lead to altered frequency of conspecific amplexus, oviposition, embryo viability, or overall reproductive success, decreases in any one of which would constitute reproductive interference. Additionally, if mechanisms of species recognition and mating preference mitigate reproductive interference and contribute to species sexual isolation and coexistence (Som et al., 2000), amplexus and oviposition should be relatively insensitive to our manipulation of social environment and exhibit a strongly nonlinear relationship

with conspecific relative abundance. Conspecific sexual activity and high reproductive success should predominate at all but low relative abundance of conspecific partners if mechanisms promote assortative mating among species and decrease reproductive interference. Based on our results, we present a descriptive model for conspecific mating (i.e., sexual isolation) as a function of social environment. We then discuss this relationship in the context of mate identification, male-male competition in anurans, and the ecology and conservation of *R. latastei*.

METHODS

Study system and natural history

Rana latastei is a species of international conservation concern (listed as “nearly threatened” by the IUCN Species Survival Commission, 2000), and, in Switzerland, it is red-listed at the highest level of endangerment (Grossenbacher, 1994). Females deposit one clutch per year, and oviposition occurs over just a few seconds (Hettzey A and Pearman PB, personal observations). Over much of its range (northern Italy, western Croatia and Slovenia, and extreme southern Switzerland; Grossenbacher, 1997a; Societas Herpetologica Italica, 1996), *R. latastei* lives in frequent syntopy with *R. dalmatina*. The two species both breed from early February through late March, depending on elevation, and their relative abundance at breeding sites in Switzerland varies greatly (Grossenbacher K, unpublished data; Pearman PB, unpublished data). *R. latastei* exhibit aggregated oviposition in cool, shady ponds and streams in and near forest, their preferred habitat (Ildos and Ancona, 1994), and clutches accumulate in tight aggregations on submerged vegetation.

R. dalmatina, in contrast, deposit clutches scattered throughout sunny, shallow lentic habitat, attached to twigs or other vegetation (Nöllert and Nöllert, 1992). Habitat segregation between the species is incomplete, and they both reproduce in ponds and drainage ditches near forest edge. Where *R. latastei* is scarce relative to *R. dalmatina*, contributing 10% or less of all brown frog clutches, 50% or more of the *R. latastei* clutches may be unfertilized or die very early in development. Heterospecific amplexus between these frogs occurs naturally (Grossenbacher, 1997b). Furthermore, male *R. dalmatina* demonstrate agonistic behavior with *R. latastei* males, attempting to displace them from conspecific amplexus (Pearman PB, personal observation). These observations suggest that high relative densities of *R. dalmatina* may lead to interference with reproduction by *R. latastei*.

Experimental treatments and arenas

If *R. dalmatina* have no impact on the mating behavior of *R. latastei*, changing the relative abundance of the two species should not affect the frequency with which *R. latastei* amplex with conspecifics. To detect interspecific interaction and changes in the reproductive success of *R. latastei* females, we provided single *R. latastei* females with *R. latastei* males and *R. dalmatina* males, at four relative abundances and a single overall density of six males. There were one, three, five, or six *R. latastei* males present, corresponding to ratios of 1:5, 3:3, 5:1, and 6:0. This density was within the range of local densities of males in natural ponds. Under the hypothesis of no reproductive interference, the expected frequency of matings between conspecifics is 1.0 for all four treatments. We observed amplexus among individuals and recorded clutch deposition and viability. This design had the advantage of not confounding the relative abundance of either species with the total density of all potential sexual partners and was sufficient

for detecting interspecific interference. It did not, however, allow us to exactly determine the relative contribution of variation in the absolute density of *R. latastei* males to embryo viability or relative reproductive success (see Discussion).

Eight inflatable plastic wading pools (120 cm × 120 cm, 60 cm deep) were placed in a field, filled to a depth of 25 cm with pond water, and left uncovered. To provide standard substrates for egg-deposition, we placed a plastic foam brick holding three wooden dowels (4 mm thick, 0.5 m long, 45° from vertical) in three corners of each pool. In preliminary experiments, *R. latastei* oviposited more readily when dowels had been pulled through a *R. latastei* egg clutch, consistent with *R. latastei*'s aggregated oviposition in nature. We pulled the dowels through *R. latastei* clutches before the experiments to simulate sites of active oviposition.

Experimental animals

We hand-collected individuals of both *R. latastei* and *R. dalmatina* from two active breeding sites in the Mendrisiotto, Canton of the Tessin, Switzerland, on several evenings of February and March 2001. The species were syntopic at these two sites. From one site ("Seseglio," on Swiss topographical series map for Mendrisio, 1:50,000), we collected 80 *R. latastei* males, 64 *R. latastei* females, and 41 *R. dalmatina* males. From the other site ("Ca del Boscat") we captured 1 *R. latastei* male, 3 *R. latastei* females, and 28 *R. dalmatina* males. *R. latastei* from these populations exhibit no substantial genetic divergence (Garner et al., 2003). Females and males were then transported in separate, covered plastic containers (57 cm long × 37 cm wide × 31 cm deep) to the site of the experiment, about 3 km away. Animals were held in fresh pond water at ambient temperature.

Males and females of both species were collected from active choruses. We further examined each male for well-developed nuptial pads, as indicators of readiness for reproduction. We sorted males by species, measured snout-vent length, and marked each individual with a numbered waistband to allow identification. To reduce possible bias caused by repeated use of dominant and/or exhausted males, we excluded individuals that achieved amplexus and remained until oviposition from further experiments. Females were examined manually for eggs. We used each female only once during the experiment, and males were used maximally four times. We returned used males and females to the site of their collection.

Experimental protocol

The experiments were performed from 23 February to 20 March 2001, starting in the evening at about 2100 h. Immediately before each replicate of the experiment, the four social environments, as well as all male frogs, were randomly assigned to arenas in two spatial blocks using computer-generated, pseudorandom numbers. We allowed males to acclimatize for 15 min. To begin a replicate of the experiment, we then placed a randomly chosen female into the middle of each pool and recorded the starting time. We monitored the arenas regularly for the first 3 h, during which time we observed each arena for 1.5 min every 15 min. We recorded which male (if any) was in amplexus with the female, all males attempting to amplex a free female, males attempting to displace an amplexant male, and which male was in amplexus when the female oviposited. At 0900 h the next morning we noted in which arenas clutches had been laid. In the arenas without clutches, we recorded whether an amplexant pair was present and continued observations for an additional 3 h (for a maximum total of 15 h in absence of

oviposition), at which point we terminated the replicate. On one hand, limiting observation to 3 h in the evening and 3 h the next morning allowed us a high level of replication during the short breeding season. On the other hand, it limited our ability to identify the male in amplexus at oviposition.

Total counts of eggs were not possible. *R. latastei* clutches are a tight mass of eggs and must be cut apart to be counted. Because of the endangered status of the species, we were obligated to return clutches to breeding ponds. An observer ignorant of treatment of origin estimated the percentage of viable embryos between 48 and 72 h after oviposition. Total inviability was scored as zero. Clutches with viable eggs were assigned scores corresponding to the following upper limits of intervals: 5%, 10%, 20%, and by increasing intervals of 10%, up to 90%, followed by 95% and 100%. Counts of four sample clutches demonstrated good consistency.

Response variables and statistical analysis

We compared amplexus of the two species and the reproductive success of *R. latastei* among the four treatments using four measures: (1) time to first amplexus with any male; (2) type of mating at first amplexus (heterospecific or conspecific); (3) clutch laying (clutch deposited or not); and (4) embryo viability, determined as the proportion of developing embryos per clutch. We chose these variables because they represent distinctly different aspects of reproductive behavior. Amplexus and oviposition are also unmistakable events, offering little opportunity for the intrusion of observer bias where double-blind trials are not feasible. Additional variables would have been conceptually redundant and/or correlated with these four, thus increasing the ratio of response variables to observations and the chances of inflated Type I error.

We analyzed data on elapsed time until first amplexus using Cox regression, a type of survival analysis (Allison, 1995). We included observations where amplexus occurred within the first 3 h, being assured of observing initial amplexus events. Additional observations in which clutches were deposited but amplexus was not observed or in which males unsuccessfully attempted to enter amplexus or amplexus was observed only the next morning were included and considered right censored. Observations with neither male-female interaction nor oviposition were omitted from this analysis.

Count data describing the type of mating at first amplexus and the number of units in which clutch laying occurred were each tabulated with respect to treatment. We tested for an effect of treatment on these variables using Fisher's Exact test, as implemented in SAS Proc Freq, because some table entries had values < 5 (SAS Institute, 1988). Differences in embryo viability among treatments were tested for significance using a Kruskal-Wallis test as implemented in SAS Proc NPAR1WAY because residuals from ANOVA resisted transformation to normality (SAS Institute, 1988; Siegel and Castellan, 1988).

RESULTS

Measurements of male *R. dalmatina* snout-vent length (SVL) average 57.0 ± 0.70 mm (SE, $n = 58$) whereas *R. latastei* males measure 50.7 ± 0.31 mm ($n = 81$), a significant difference (Kruskal-Wallis $\chi^2_3 = 42.7$, $p < .0001$). We found no evidence for systematic differences in SVL among treatments for either *R. dalmatina* males (Kruskal-Wallis $\chi^2_2 = 1.51$, $p = .47$) or for *R. latastei* males (Kruskal-Wallis $\chi^2_3 = 2.42$, $p = .49$). We observed no evidence of SVL differences between mated and unmated males within the replicates of the 6:0 treatment (pairwise comparison of amplexant male vs. average of nonamplexant males; Wilcoxon signed-ranks test, $p > 0.4$, n

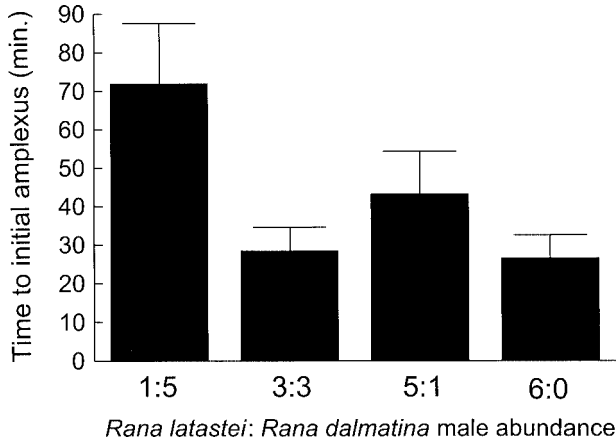


Figure 1
Time to first amplexus. Column height indicates average time for four relative abundances of *Rana latastei* males in artificial ponds used as breeding arenas. Bars indicate 1 SE.

= 13). We also found no evidence for systematic variation in female SVL among treatments ($F_{3,11} = 0.43, p = .73$).

Average time to first amplexus varied with relative abundance of conspecific males and was longest in the 1:5 treatment (Figure 1). Under our criterion for inclusion in survival analysis, 14.6% of the observations were right censored. Using three dummy variables to represent observations in the 3:3, 5:1, and 6:0 treatments, we used the TEST option in SAS Proc Phreg to test two independent hypotheses (Allison, 1995). First, we found statistical support for a difference in time to amplexus between the 1:5 and 6:0 treatments (Wald $\chi^2_1 = 6.69, p = .01$). However, second, there was no evidence for differences in the time to amplexus among the 3:3, 5:1, and 6:0 treatments (Wald $\chi^2_2 = 3.05, p = .21$).

The type of mating (heterospecific or conspecific) at initial amplexus depends on the relative abundance of *R. latastei* and *R. dalmatina* males in the experimental arenas. Amplexus among heterospecific males and females was observed in all treatments where *R. dalmatina* was present. Nine of 10 initial amplexes were conspecific in both the 5:1 and 3:3 treatments, but only 1 of 9 initial amplexes was between conspecifics in the 1:5 treatment. A test of the corresponding two-by-three contingency table was significant (Fisher's Exact test, $p < .001$), indicating that amplexus of females with conspecifics depended on the presence and relative abundance of *R. dalmatina*. The low rates of initial conspecific amplexus in the 1:5 treatment were nearly identical with that expected under random mating (1.6:8.4 vs. 2.0:8.0, expected vs. observed, respectively), as was the frequency of conspecific amplexus in the 5:1 treatment. However, in the 3:3 treatment, initial amplexus among conspecifics was more frequent than the expectation of 50% conspecific amplexus ($\chi^2 = 6.4, p = .0121$, Bonferroni-corrected $\alpha = 0.016$). This indicates a strongly nonlinear behavioral response with changing relative abundance of conspecifics and shows that conspecific pairings were favored in the 3:3 treatment.

Oviposition frequency of *R. latastei* females during 15 h of observation increased from 25% to 50% with increasing relative abundance of conspecific males. Oviposition was recorded in 4 of 16 replicates, 5 of 17, 6 of 16, and 9 of 18 replicates in the 1:5, 3:3, 5:1, and 6:0 treatments, respectively. We observed that 22 of 24 clutches were deposited after the initial 3-h observation period.

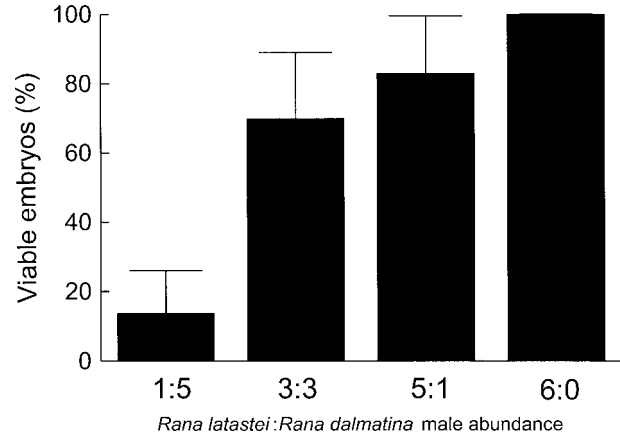


Figure 2
Mean embryo viability (+ SE) for clutches deposited under four conditions of relative abundance of potential conspecific mates ($n = 5, 6, 8, 9$ for the 1:5, 3:3, 5:1, and 6:0 treatments, respectively).

The mean proportion of viable embryos in clutches was greatest in the 6:0 treatment and least in the 1:5 treatment, $100\% \pm 0\%$ and $13.6\% \pm 12.1\%$, respectively (mean \pm SE; Kruskal-Wallis $\chi^2 = 12.6, df = 3, p < .006$, Figure 2). Except for the 6:0 treatment, distributions of viability were highly bimodal. In the 5:1 treatment, five clutches showed 100% viability and three showed 0%. In the 3:3 treatment, three clutches demonstrated between 90% and 100% viability, two demonstrated 0%, and one had 60%. In the 1:5 treatment, four demonstrated 0–5% viability, and one had 50% viability. The difference in mean viability between the two extreme treatments was significant (Kruskal-Wallis $\chi^2 = 11.5, df = 1, p < .001$, Bonferroni-corrected $\alpha = 0.0083$, six comparisons). Other post-hoc comparisons between treatments were not strongly significant when the correction in significance level was made to account for multiple comparisons (all $p > .02$).

Mean embryo viability declined with decreasing abundance of conspecific males in the restricted set of units in which oviposition was recorded after observation of conspecific amplexus. In the 5:1 treatment, four females were recorded as producing clutches during the night, after being last observed in amplexus with a conspecific male. These clutches all had 100% embryo viability. In the 3:3 treatment, five females were recorded as producing clutches after last being observed with a conspecific. These five clutches had an average viability of 70% ($\pm 19\%$). Of the four clutches oviposited in the 1:5 treatment, one occurred after a *R. latastei* male was last seen with the female. This clutch presented embryo viability of 50%.

Within the restrictive conditions of this experiment, the data allowed a first approximation of fecundity *R. latastei* females in the 1:5, 3:3, and 5:1 treatment relative to females in the 6:0 treatment, based on the unreplicated estimate of the probabilities of oviposition and embryo viability. We assumed that the observed frequency of clutch deposition and of embryo viability represented independent probabilities of oviposition and viability, and that clutch size did not differ between the treatments. By way of an example, the ratio of realized fecundity, $\lambda_{1:5}/\lambda_{6:0}$, is the ratio of two products

$$\frac{\lambda_{1:5}}{\lambda_{6:0}} = \frac{o_{1:5}v_{1:5}}{o_{6:0}v_{6:0}}, \quad (1)$$

where $o_{1:5}$ and $o_{6:0}$ are probabilities of oviposition of *R. latastei* in the low and high abundance treatments (0.25 and 0.5, respectively), and $v_{1:5}$ and $v_{6:0}$ are average embryo viabilities

(0.136 and 1.0, respectively). *R. latastei* females in the 1:5, 3:3, and 5:1 treatments averaged 6.8%, 41%, and 62% the success of females in 6:0 treatment. Multiplication of the relative abundance of conspecific males by the success observed in the 6:0 treatment gives expected relative reproductive success under random mating of 8.3%, 25%, and 41% in the same three treatments.

DISCUSSION

Variation in population vital rates, such as birth rate here, is closely linked to the dynamics of populations and probabilities of colonization and extinction (Hassell et al., 1976; Richter-Dyn and Goel, 1972). The relationship between social environment and among-population variation in reproductive success suggests that studies of behavioral responses to social environment can contribute to understanding the relationships among environment, among-population variation in behavior, and population dynamics (Anholt, 1997). The tendency of some anurans to amplex with heterospecifics and inanimate objects is well-known to herpetologists and selects for convergent release calls among species (Leary, 2001). Although the potential effect of heterospecific amplexus on demography via reproductive interference in mixed species systems has obvious intuitive appeal, the effect of variation in behavior on population vital rates has rarely been demonstrated experimentally. Greater than expected sexual isolation in the 3:3 treatment suggests that one or more unidentified mechanisms, such as mate recognition by males or female preference for characteristics of conspecifics (Ryan and Rand, 1993), function in maintaining sexual isolation between these species and reducing interference. Female preference and assortative mating among species can in theory contribute to species coexistence (Som et al., 2000).

Substantial decrease in sexual isolation ($a < 1.0$; Figure 3) indicates that manipulation of the social environment here leads to conditions likely to produce reproductive interference. When conspecific mates are relatively infrequent (the 1:5 treatment), conspecific amplexus is inhibited, and amplexus frequency among conspecifics closely approximates that expected under random mating. This behavioral pattern suggests that the high relative abundance of heterospecifics disrupts sexual-isolating mechanisms. Reduced relative abundance of conspecifics is concurrent with reduced oviposition frequency. But in spite of the tendency for initial amplexes to be among conspecifics in all but the 1:5 treatment, there was a tendency for embryo viability to decrease with decreasing conspecific abundance. This may suggest that choice mechanisms and mate identification, while promoting sexual isolation, do not completely compensate for processes leading to reproductive interference. Even when conspecific amplexus occurs, embryo viability is reduced in the clutches that are eventually deposited when conspecific mates are rare. This suggests that conspecific amplexus by *R. latastei* is not sufficient to prevent effects of reproductive interference on embryo viability and overall reproductive success in the presence of *R. dalmatina*, and it is consistent with observations of the two species interactions at natural breeding sites.

An alternative explanation for decreased embryo viability is that variation in absolute density of *R. latastei* males, and not reproductive interference, is fully responsible. Although this is possible, we believe it is unlikely given preferential amplexus among conspecifics in the 3:3 treatment, indicating a sexual-isolating mechanism, and given the breakdown of sexual isolation that we observed in the 1:5 treatment. In this study, reversion to random mating in the 1:5 treatment is indicative of a nonlinear decrease in sexual isolation between the 3:3 and 1:5 treatments. Decreases in embryo viability and

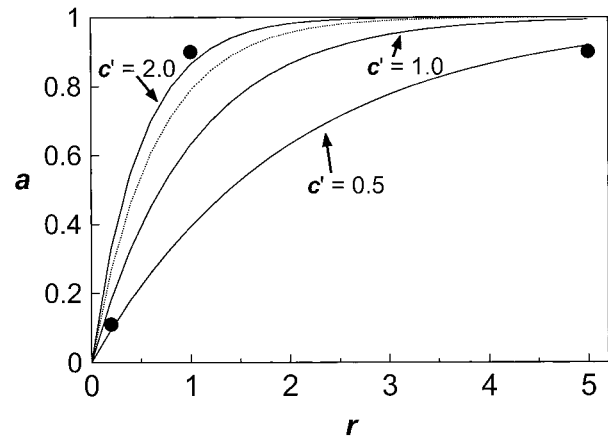


Figure 3

Proportion of amplexes among conspecifics (a) as a function of the relative abundance of conspecific and heterospecific males (r). Lines show effect of varying c' , which represents the preference of females for conspecific males, c , divided by the scaling term m in Equation 2. Here m has a value of unity. The dashed line is the function in Equation 2 ($c' = 1.56$), fitted by least squares to the data from three experimental treatments, shown as points.

relative reproductive success track this nonlinear pattern. Conversely, the maintenance of sexual isolation in the 3:3 treatment likely contributes to a rate of relative reproductive success that exceeds the rate expected under the assumption of random mating. Thus, it is unlikely that changes in the absolute density of conspecifics fully explain the patterns in viability and reproductive success we observed.

Our results also support the prediction that the initial relative abundance of species can contribute to determining persistence in systems where reproductive interference operates (Kuno, 1992). The patterns we observed suggest an Allee effect of relative abundance on sexual isolation and resulting reproductive success. Further, they support a model in which sexual isolation between *R. dalmatina* and *R. latastei*, and the latter's reproduction, collapse when the latter species is at low relative abundance. The collapse of sexual isolation and the decrease in reproductive success in the 1:5 treatment are sufficient to explain our observation of *R. latastei*'s occasional reproductive failure in the field.

Natural history observations and the present results on reproductive interference suggest a nonlinear, frequency-dependent model for sexual isolation between *R. latastei* and *R. dalmatina*. We propose the following model for sexual isolation as a function of social environment,

$$a = 1 - \frac{e^r}{e^m} \quad (2)$$

In this model a is the proportion of conspecific matings (range: 0 – 1), e is the base of natural logarithms, and c is a preference coefficient describing the degree to which females prefer conspecific males over heterospecific males (under unspecified standard conditions) and which does not vary with relative abundance of conspecifics. The variable r is the ratio of conspecific males to heterospecific males. The scaling parameter m relates the experimental determination of female preference, c , to observed amplexus rates under conditions specific to a mixed-species breeding chorus, thus accounting for differences among the species in levels of male–male competition among conspecifics and propensity for interspecific interference by heterospecifics. Varying c/m and r produces curves that vary in the linearity of the effect of social environment on the proportion of conspecific

amplexes (Figure 3). Increasing nonlinearity represents increasingly effective sexual-isolating mechanisms, which may reduce reproductive interference. These curves share the characteristics of producing no conspecific amplexus when all males are heterospecifics ($a = 0$) and of asymptotic approach to unity (no mating with heterospecifics) with increasing ratio of conspecific to heterospecific males.

Interference and reproductive success

Reduced embryo viability may result when *R. dalmatina* males displace *R. latastei* males at oviposition or when *R. latastei* females eventually oviposit after initial amplexus by *R. dalmatina* males and fail to produce viable embryos. The absence of viable embryos after amplexus of *R. latastei* by *R. dalmatina* is consistent with several ad hoc attempts to artificially fertilize *R. latastei* eggs with *R. dalmatina* sperm (Hettzey A, unpublished data). Additionally, genetic studies of embryos in *R. dalmatina* clutches from nature indicate multiple paternity (Holveck M-J, unpublished data). This result and observation of direct “amplexus” by male *R. dalmatina* with both recently oviposited conspecific clutches and clutches of *R. latastei* (Grossenbacher K, unpublished video recording) suggest that sperm competition may contribute to reduced viability of *R. latastei* clutches. Our estimate of differences in reproductive success under extremes of relative abundance of conspecific males may be an approximation specific to the experimental conditions we established. There is, nonetheless, no indication that later matings are primarily among conspecifics or that a longer observation period would alter our conclusions.

Female discrimination of conspecific males from heterospecifics may be weakened when opportunities to compare conspecifics and heterospecifics are limited, as occurs when conspecifics are relatively infrequent, distant, or displaced temporally (Michalak and Rafinski, 1999; Verrell, 1990; Wells, 1977). In the present study, we observed increased time to first amplexus when conspecific males were infrequent, suggesting a reluctance to form amplexus (Figure 1). This may, in part, be a function of choice by *R. latastei* females. Another contributing factor may involve preferences expressed by *R. dalmatina* males, which may also differ from *R. latastei* males in their propensity to amplex with *R. latastei* females. Further, male–male competition may play a role in determining access to females (Berven, 1981) and can overwhelm female preferences (Reyer et al., 1999). Sufficient male–male competition may explain why sexual isolation breaks down when conspecifics are at low relative abundance. Alternatively, the fact that the frequency of conspecific amplexus in the 1:5 treatment is close to that expected based on random mating suggests that the presence of heterospecifics may passively interfere with species recognition by *R. latastei*, resulting in a random outcome.

Reproductive interference, sexual isolation and demography

Progress in linking social behavior and demographic characteristics of populations could be made by addressing additional environmental factors that impact sexual isolation between populations, then examining the demographic results. Sexual isolation and mate choice are seen as two sides of the same coin from an evolutionary perspective (e.g., Lande, 1982; Ryan and Rand, 1993). However, the relationship between sexual isolation, or its failure, and population demography is poorly developed, in contrast to the relationship between sexual isolation and the production of hybrids. Some repercussions of breach of sexual isolation are a recognized source of demographic phenomena (Rhymer and Simberloff, 1996). Extinction through introgression, for

example, depends on the breakdown of sexual isolation and has been shown in simulation models to depend additionally on environmental factors, such as species relative abundance and opportunities for habitat segregation (Epifanio and Philipp, 2000; Wolf et al., 2001).

In experimental studies, sexual isolation may depend on environment during ontogeny (Brazner and Etges, 1993), the degree of lineage divergence between interacting species (Arnold et al., 1996), and opportunities for females to compare conspecific and heterospecific males (Michalak and Rafinski, 1999). Several additional mechanisms can produce reproductive interference as a result of variation in social environment (e.g., distraction of males by heterospecifics: Singer, 1990; Schultz and Switzer, 2001; interspecific aggression: Alatalo et al., 1994; brood parasitism: Sorenson, 1997). Nonetheless, the breakdown of sexual isolation may be unique as a mechanism of reproductive interference because it can involve conflicting selection for choice of a high-quality partner, male competitive ability, and capacity for species recognition (Reyer et al., 1999; Pfennig, 2000).

The results of the present study suggest that a relationship among behavioral variation, sexual isolation, and demographic rates (here birth rate) can depend on social environment, one component of which is the structure of mixed-species breeding assemblages. The occurrence of reproductive interference and incomplete habitat segregation suggests the importance of conserving and restoring forested sites in the river bottom land that is favored by *R. latastei* throughout the Po River basin. Forest restoration may increase opportunities for habitat segregation between *R. latastei* and congeners, altering the frequency of social environments. This may potentially reduce levels of reproductive interference from what may occur in forest-edge sites where syntopic reproduction dominates.

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