

COMMENTARY

Testing Experimental Results in the Field: Comment on Ficetola and De Bernardi (2005)

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Ficetola & De Bernardi (2005) test the effects of reproductive interference between *Rana latastei* and *Rana dalmatina* on the former's reproductive success under natural conditions. Their study examines predictions by Hettyey & Pearman (2003), based on natural history observations and experimental results, that reproductive failure may occur because of heterospecific matings when species relative abundance is highly skewed. Ficetola & De Bernardi (2005) measure *R. latastei* reproductive success, represented by mean embryo viability across clutches raised in the laboratory, using three populations that are syntopic with *R. dalmatina* and two other populations, where this congener is absent. Ficetola and De Bernardi find no decrease in reproductive success of *R. latastei* populations in syntopy. They also find no relationship between the relative abundance of heterospecifics and *R. latastei* reproductive success. Ficetola & De Bernardi (2005) conclude that heterospecific individuals do not interfere with *R. latastei* reproduction under natural conditions and they emphasize validating experimental results using natural populations.

We agree with Ficetola & De Bernardi (2005) that tests of experimental results under natural conditions are needed. Determining an optimal experimental setup is difficult because different setups may yield different results (e.g. Michalak & Rafinski 1999). Further, influential factors in experimental manipulations may be unimportant under natural conditions (e.g. Skelly & Kiesecker 2001). In this sense, the aim of Ficetola & De Bernardi (2005) to test the validity of experimental results using natural populations is admirable. Nonetheless, correlative tests of experimental studies must be designed with statistical rigor and a thorough understanding of the original studies

in order to provide valid tests of predictions. Ficetola & De Bernardi (2005) is flawed in this respect.

The design in Ficetola & De Bernardi (2005) creates two biases toward accepting the null hypothesis that reproductive interference by *R. dalmatina* does not affect *R. latastei* in nature. Firstly, Ficetola & De Bernardi (2005) ignore the result that the relationship between species relative abundance and effectiveness of sexually isolating mechanisms is strongly non-linear (Hettyey & Pearman 2003). Abundance of *R. latastei* and *R. dalmatina* in the syntopic study populations of Ficetola and De Bernardi is estimated to be 1:1, 1:2, and 1:5. Hettyey & Pearman (2003) observe no significant decrease in fertilization success in trials, where *R. latastei* males are not outnumbered by *R. dalmatina* males by at least 1:5, probably because of the mitigating effect of mate recognition mechanisms. The results and model of Hettyey & Pearman (2003) predict significant decrease in average reproductive success in nature only when one species occurs at very low relative abundance. Decreased reproductive success where *R. latastei*'s relative abundance is 1:1 and 1:2 is not expected, thus grouping all syntopic populations together biases Ficetola and De Bernardi's test. Further, absence of a bimodal distribution of embryo viability and no decrease in embryo viability in the one population where *R. latastei* was outnumbered by 1:5 would suggest that conspecific–heterospecific ratios be <1:5 in some natural populations to disrupt sexual isolation. However, one cannot draw such a conclusion from the single population in Ficetola & De Bernardi (2005) in which the phenomenon was expected.

Secondly, Ficetola & De Bernardi (2005) suffers from small sample size, which biases the study

toward the null result. They investigated only two populations in allotopy and three in syntopy and themselves report extremely low power (0.057) in a post hoc analysis. Their Fig. 2 is inconclusive because of their small sample. In fact, average fertilization success in the three syntopic populations was lower than in one of the two allotopic populations, actually supporting Hettyey & Pearman's (2003) prediction of decreased hatching success in syntopy.

Additionally, the two allotopic populations originate in a different area than the three syntopic ones, although broad overlap of the species ranges makes this confounding unnecessary. Further, the selection of study populations had no random component. Thus, the degree to which these populations represent *R. latastei* populations in general is questionable. Unquantified spatially confounded factors may mask differences in the fecundity or behavior of males, or variation in sex ratio among populations may impact the potential for reproductive interference (Foster 1999). For example, Ficetola & De Bernardi (2005) estimated the abundance of the two interacting species solely by counting egg-clutches, thus assuming sex ratios within species are similar in all populations. The possibility of significant among-population variation in sex ratios cannot, however, be excluded. Sex ratio variation may mask the effect on *R. latastei*'s reproductive success that is exerted by the species' relative abundance because these two factors together determine the heterospecific/conspecific male ratio. Finally, while Ficetola & De Bernardi (2005) cite results on neutral genetic markers to support existence of a relevant fitness gradient (Garner et al. 2004), the necessary data are absent concerning this potentially important population characteristic.

Ficetola & De Bernardi (2005) argue that unnatural experimental conditions in Hettyey & Pearman (2003) may alter the behavior of *R. latastei* and *R. dalmatina* males asymmetrically and lead to experimental artifacts. In the current context, all successful experimental manipulations alter animal behavior. Nonetheless, inspection of Hettyey & Pearman's (2003) field experiment reveals no species differences in ability to cope with stress. If *R. dalmatina* were to have such an advantage, breeding *R. latastei* females to three *R. latastei* and three *R. dalmatina* males would cause a statistically significant drop in

reproductive success in comparison with breeding with six conspecifics. This was, however, not observed. The data do not support a stress tolerance hypothesis (Hettyey & Pearman 2003).

Sample size and other aspects of design in Ficetola & De Bernardi (2005) encumber interpretation of their results. Adequate numbers of syntopic sites, selected with attention to their distribution, sufficient randomization and the species' relative abundance are important. Further, productive tests of predictions in Hettyey & Pearman (2003) might focus on the adequacy of relative male abundance and two parameters describing female preference and environmental effects for modeling sexual isolation in nature. One approach would be to address site-specific factors that potentially influence the breakdown of sexual isolation, as some *R. latastei* behaviors (e.g. aggregated oviposition) may contribute to it and testable models for sexual isolation exist. Careful translation of the predictions of Hettyey & Pearman (2003) to natural systems will help illuminate the effects of reproductive interference between *R. latastei* and *R. dalmatina* specifically, and the importance of behavioral variation for population vital rates and dynamics in general.

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