THE EVOLUTION OF GAMETIC INCOMPATIBILITY IN NEOTROPICAL *ECHINOMETRA*: A REPLY TO MCCLARY

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In an earlier paper (Lessios and Cunningham 1990), we examined gametic compatibility among three species of the sea urchin genus *Echinometra*. Two of these species (*E. lucunter* and *E. viridis*) are found in the Caribbean, whereas the third (*E. vanbrunti*) was isolated in the eastern Pacific by the Pliocene rise of the Isthmus of Panama. We found that crosses between *E. viridis* and *E. vanbrunti* produced rates of fertilization almost equal to those involving homogametic crosses, and that sperm of *E. lucunter* could fertilize eggs of the other two species at comparable rates. However, the eggs of *E. lucunter* could not be fertilized by heterospecific sperm. This barrier was significantly more effective toward the allopatric species than toward the sympatric one. On the basis of the four possible phylogenetic topologies linking the three species and on the known date of completion of the Central American Isthmus, we discussed the implications of this finding regarding the evolution of reproductive isolation in marine invertebrates with external fertilization. McClary (1992) wrote a critique of this article centered on two issues, one conceptual, the other technical. His two points are that "examination of gamete compatibility can provide an important insight into the evolution of closely related species, but cannot solely be used to infer phylogenies" (p. 1580) and that "exposing eggs to a high concentration of spermatozoa is highly artificial" (p. 1579).

We are puzzled by McClary's claim that we have used gametic compatibility to infer phylogeny, because our article indicates quite clearly that we did not. We discussed the probability that each possible phylogenetic topology was correct in the context of previous evidence on morphological and isozymic divergence between these species (Lessios 1979, 1981), placed the emer-

gence of gametic isolation on each tree, and concluded that "there is no correlation between reproductive isolation and existing measures of genetic divergence within the genus (Lessios and Cunningham 1990, p. 938). In direct contradiction to McClary's assertion, the only topology we rejected (as supported by neither morphology nor isozymes) is the one that would have been suggested by gametic compatibility, that is, an ancient origin of *E. lucunter* and a more recent split between the two gametically compatible species. McClary stated that "the topology which appears to be favored by the authors, that *E. viridis* split from the stock that would eventually result in the two geminate species, *E. lucunter* and *E. vanbrunti*, before the rise of the Isthmus (Fig. 1D, p. 937) depends on the assumption that compatibility between gametes, once lost in evolution, is unlikely to be regained" (p. 1579). Why this topology should depend on any assumption regarding gain and loss of the ability to cross-fertilize is entirely unclear. We do not see how it is logically possible to believe simultaneously that we favor an early split of *E. viridis* and that we used gametic compatibility to infer phylogeny. Our article indicates that we did neither. Thus, with respect to the question of whether phylogenies can be built on the basis of data on gametic compatibility between species, there is no disagreement between our views, as presented in Lessios and Cunningham (1990), and those of McClary (1992).

Although we did not use it to build phylogenetic trees, we did make the assumption that compatibility between eggs and sperm of the populations once lost in evolution is not likely to be regained, and we did state that if this assumption were true, the apparent compatibility between gametes of *E. vanbrunti* and *E. viridis*

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could not have been secondarily acquired. Is such an assumption reasonable? In Lessios and Cunningham (1990), we cited studies indicating that the affinity between "bindin" of the sperm and "bindin receptors" of the egg is species-specific and depends on their chemical composition. We also pointed out that given this chemical specificity, the coding involved in recognition between gametes could be lost by accidental mutations but would be unlikely to be restored by the same process. Instead of considering this evidence or our reasoning, McClary cited studies in which laboratory experiments under various conditions and treatments of the gametes have produced varying percentages of fertilization between "both closely and distantly related" echinoid species. McClary's reasoning is not stated explicitly, but he presumably assumed that the distantly related species have regained gametic compatibility that their ancestors had lost. It is not obvious why this should be so. The most parsimonious explanation for the ability of these species to cross-fertilize each other is that they never lost gametic compatibility because "decoupling of the emergence of reproductive isolation from genetic divergence may be common" (Lessios and Cunningham 1990, p. 939). In the absence of information regarding the phylogenetic relationships of the species involved in the studies cited by McClary, we see no reason to believe that the complex molecular message involved in gametic recognition was scrambled and then accidentally unscrambled. Even if the assumption of unidirectional evolution of gametic incompatibility were eventually proved wrong, the only conclusion in Lessios and Cunningham (1990) that would need to be reconsidered is that the barrier to fertilization by heterospecific sperm of *E. lucunter* eggs evolved after it became separated from the other two species.

The technical point raised by McClary is that the concentration of 5×10^8 sperm mL^{-1} in our experiments was "relatively high" and that it would have been better to have used a lower ratio of sperm to eggs. However, the sperm concentration we used is very close to that found by others to be necessary for 100% fertilization of conspecific eggs (e.g., Levitan et al. 1991). Simple kinetic considerations and experimental evidence (Levitan et al. 1991) indicate that the concentration of eggs is unimportant. Because McClary believes that the success of fertilization depends on the ratio of spermatozoa to eggs, rather than absolute sperm concentrations, he

expressed concern that the ratio of $10^5 : 1$ that we used could have resulted in one of two possible artifacts: It might have overwhelmed interspecific barriers, or alternatively it might have resulted in "the induction of physiological blocks to polyspermy by the egg, reducing the fertilization success rate" (p. 1579). In this, he has ignored that we employed homogametic controls performed with gametes from the same animals in the same concentration as those of heterogametic ones, and that we excluded any experiments that gave us less than 70% of cleaving eggs in either of the two homogametic crosses. He has also disregarded that we quantified the percentage of both fertilized and cleaving eggs in each experiment and found the two to be approximately equal, which makes it obvious that polyspermy was not a factor. Most important, he has failed to consider how each possible artifact would have affected our findings. If the apparent barrier to fertilization by heterospecific sperm of *E. lucunter* eggs were not due to natural incompatibility but came from blocks to polyspermy, how does one explain that eggs from the same parents did not develop such blocks when exposed to the same concentration of conspecific sperm? And why did the other two species, also exposed to the same concentration of sperm, produce rates of fertilization and cleavage close to 100%? Conversely, if the sperm concentrations we used could overwhelm barriers to interspecific hybridization, how can our finding regarding *E. lucunter* eggs be explained?

McClary contradicted his own assertion that gametic compatibility should not be used in phylogenetic reconstruction when he stated that varying concentrations of eggs and sperm in each experiment would "allow for more accurate phylogenetic inferences to be made" (p. 1579). Nevertheless, he was correct in thinking that such fertilization profiles would have provided information on the dynamics of sperm-egg interactions. For example, the sperm concentrations we used may have obscured weaker incompatibility between heterospecific sperm and the eggs of *E. vanbrunti* or *E. viridis*. That some discrimination by heterospecific gametes of these species exists is obvious in our data in the form of statistically significant differences in fertilization and cleaving rates of heterogametic versus homogametic crosses. However, as we acknowledged when we rejected the biological importance of these reductions, the evolutionary interest lies in strong barriers to heterospecific fertilization that would

actually (in the case of sympatric species) or potentially (in the case of allopatric ones) prevent gene flow between the species, and thus serve as isolating mechanisms. We used sperm concentrations necessary to fertilize all eggs in intraspecific controls precisely because we were interested in such strong barriers. That other mechanisms of reproductive isolation, in addition to the protection of *E. lucunter* eggs, exist between the sympatric species and may also exist between allopatric ones is undeniable and acknowledged in our article. A study of the kinetics of fertilization in homogametic and heterogametic crosses would have involved many more variables than McClary's ratio of sperm to egg concentrations, the most important being the time of exposure of eggs to sperm (e.g., Levitan et al. 1991). Such a study would be interesting on its own right, but its results would be unlikely to affect our conclusions regarding the evolution of gametic incompatibility in neotropical *Echinometra*.

We conclude that McClary's critique is unfounded. His conceptual "disagreement" was generated by his misunderstanding of our article; his technical objection, though correct in a general sense, does not apply to the evolutionary question we asked or the conclusions we reached.

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