

Reviews of MBE paper

Reviewer #1

Over the past 15 years Vacquier and his students have made lysin the best-understood fertilization protein on earth from an evolutionary perspective. The mode of action of the protein is understood, its role in reproductive isolation has been documented, and even its 3-dimensional structure has been worked out. One of the fascinating aspects of this protein is its rapid evolution, an evolution that appears to have been driven by selection. In an attempt to understand the nature of the selection that promotes divergence of lysin between species, Vacquier and Swanson have published a series of important papers on VERL, the receptor protein for lysin. Prior to their work, the assumption of most workers in the field was that the receptor would demonstrate rapid, selection-driven evolution as well. However, the story has proven more complicated. VERL has a repetitive structure, containing about 28 repeats of 153 amino acids. Although this protein gives evidence of concerted evolution, past work has failed to find signals of positive Darwinian selection. The current manuscript, which represents a collaboration between Swanson, Vacquier and Aquadro, reports on further attempts to identify the evolutionary forces affecting the divergence of VERL. In addition, the manuscript contains a verbal model which attempts to explain the rapid evolution of lysin. Finally, the manuscript presents some surprising new data indicating that pink abalone have two forms of VERL that rarely occur in the heterozygous condition. This finding suggests that the evolution of assortative mating may be in progress.

I do not have much to quibble with regarding this manuscript. It is well-written, the data are clearly presented, and although I am not an expert on using polymorphism surveys to detect the action of selection, the approaches appear sound. The conclusions support earlier work on this system: the evolution of VERL is best-explained by a neutral model. This finding raises the question of why lysin evolves so quickly. The authors answer this question by challenging the assumption that there must be a one-to-one correspondence between amino acid substitutions in VERL and in lysin. If lysin needs to undergo multiple substitutions for each substitution in VERL, lysin may have to evolve very quickly to keep up with neutral evolution in VERL. This model is not unreasonable and is worth presenting to the community of biologists served by Molecular Biology and Evolution. However, it should be noted that this model does not appear to pass its first test. That is, the divergence of VERL in pink abalone does not appear to be accompanied by divergence of pink abalone lysin

Reviewer #2

Review of ms. Swanson et al. VERL polymorphism and evolution

This is another fine study of abalone fertilization genes, providing an interesting set of new data, and attempting to use a series of expanded analytical tools to examine patterns of molecular evolution in the VERL/lysin genes. The comparative data on pink and red VERL sequences, the new data on the hydrophobic region and the tantalizing differences within populations of pink abalone easily warrant publication.

The new analyses look closely at the potential of positive selection to drive VERL vs lysin evolution, and like the previous paper by S and V, find no indication that this is so. This is a strong non-result (although it can be augmented, see below), but the ms may be making too much of it. In particular, the ms says that the new data support the previously proposed model of VERL gene conversion driven adaptation of lysin, but I don't see how. The new data show VERL is not evolving under positive selection, but say nothing about the validity of the previous, or any other, model. Evidence about the model would be very welcome, but would have to involve testing the model itself, perhaps including whether gene conversion exists, whether repeat polymorphism is consistent with neutrality, whether lysin interacts differently with VERL of different repeat structure, etc. Merely pointing out that the VERL/lysin pair are not both evolving by positive selection does not intrinsically support the authors previous models.

Besides this, there are a few additions that might be useful:

p. 10 - paragraph starting "Divergence between..." what is the point of this? What conclusions do you draw? These bits of data are interesting but not incorporated obviously in the framework of the ms.

p. 11 - The authors say that 'other loci' do not show subdivision in pinks. Evidence for this?

p. 13 - The authors could calculate Dn/Ds for the different types of VERL sequences in pinks to test for positive selection at this level. If this has been done in other contexts, it could be pointed out here for clarity. Another possibility is to use the new method proposed by Wyckoff et al (Nature 2000 on male germ line positive selection in humans) to see if it applies here (and to see if it applies to lysin, for that matter).

p. 14- The first paragraph discussed fertilization, and the 'great excess of sperm' available to eggs. What is the evidence that this occurs in nature? Recent

reviews point out the possibility that marine fertilization rates are sperm limited in other taxa. Any data for abalone? Some care should be taken here not to represent the ecological milieu as being better known than it is.

p. 14 - Just below this, the discussion says there is little selection on VERL-lysin affinity but that there is great selection on lysin to bind. This reflects a gender difference probably, but should be worded better to make the difference clear., otherwise it seems contradictory.

p. 14 - last 4 lines are repetitive.

p. 15 - discussion says subdivision is observed in carboxy-terminal. Data?

p. 15- -why can't you use cDNAs to get lysin sequences without bothering about the big introns? All Vacquier and co's earlier work was on lysins from cDNAs

p. 16 - co-evolution of VERL and lysin is suggested by correspondence in the levels of polymorphism, but clearly, this must be evaluated in the context of other loci. If other loci correspond polymorphism, is this evidence they are all co-evolving in the sense the authors imply? Or does it mean they are all governed by the same effective population size?

p. 17 - given the comments above that the model has NOT been advanced by this paper, I feel the last paragraph should be eliminated. The notion that mathematical modeling would help should be included earlier.

These few problems are easily solved, and the resulting ms, focusing on the empirical results, will be a valuable and interesting contribution.