

Thesis assessment of Andreevich Arseniy Lobov, **Gamete interaction proteins as factors of reproductive isolation of cryptic species of the genus *Littorina* Férussac, 1822**

The genus *Littorina*, a group of intertidal snails species have been extensively studied for evolutionary purposes as they show extreme variability within and between species and this has been associated to adaptive and speciation processes. In particular, nowadays it is being studied the mechanism of reproductive isolation between different pair of species or even subpopulations within species (*L. obtusata* vs *L. fabalis* or Crab vs Wave ecotypes of *L. saxatilis*). However, most reproductive isolation cases have involved mechanism of precopulatory reproductive isolation, while this thesis focused by first time in post copulatory reproductive isolation mechanisms by using several technologies as studying the DNA, RNA and proteins in several species.

GENERAL COMMENTS

The thesis is particular interesting because focus on both pre and post copulatory reproductive isolation mechanisms in several species of the genus *Littorina*. In particular the detection of one candidate reproductive isolation protein (LOSP) of putative postcopulatory effect is of especial relevance. The thesis uses most methodologies used to investigate these kind of mechanism from DNA to proteins and includes a study of estimation of selective effects at the DNA level by using the non-synonymous to synonymous ratio of SNPs polymorphisms. Perhaps the only partial criticisms is that the thesis has tried to investigate too many topics at the same time and so in in certain parts they have not enough time or resources to check deeply all alternatives. Nevertheless, the study, in general, is of high interest and their publications are expected to produce a relative high impact in the field. I comment below the different chapters in detail.

CHAPTER I: Literature review

I liked this chapter as the authors provide an excellent literature review. Actually such a review could be published as a professional review in a specialized journals (see Lobov et al. 2019). Actually, it seems the best strategy as any PhD student starts his/her project with a literature review, although it is rather difficult that such effort can be finished with a formal publication. Furthermore, they used proteomic information on most *Littorina* species in order to infer the physiological and phylogenetic relationships (see Maltseva et al. 2016 and 2020). Interesting they found several mismatch between DNA and proteomic information, which points to fast evolution of certain proteins during the formation of species. This emphasize the needs to include proteomic studies in order to infer evolutionary history and interpret adaptation processes.

CHAPTER 2: Precopulatory reproductive barriers

Copulating pairs were collected in three seasons on the east of Barents sea. They observed that certain pair of species show important pre-mating barriers to gene flow, while other pairs show apparently inefficient barriers (probably because they have post copulating mechanism contributing to reproductive isolation, see next chapter). This data although interesting and useful, including the potential reproductive isolation between several pairs of species it has not been published yet. I wonder if in this case they have used the right methodology and analyses in order to obtain the maximum information from the gathered data.

CHAPTER 3: identification of species-specific gamete interaction proteins

To identify gamete interaction proteins (involved in postcopulate reproductive isolation) they studied the proteome by 2D-electrophoresis linked to mass spectrometry of several tissues (seminal vesicles, prostate and penis) in four *Littorina* species. They observed several tissue specific proteins that were potential candidates of gamete interaction processes, some were species-specific. Particularly promising was the association of two sperm proteins with *L. obtusata* (LOSP-A and LOSP-B; see Lobov et al. 2020).

CHAPTER 4: Identification and description of the paraspermal protein LOSP

The thesis focused in the protein LOSP because it was the most promising a priori, and as it could not be identified by mass spectrometry they used the cDNA to infer the aminoacid sequence. Although homolog proteins or conservative domains were not detected under BLAST search, nor the secondary structure could be predicted based on model algorithms analysing the structural homology, using ANCHOR2 algorithm they could infer that the protein can bind to globular domains of other proteins. Then they used the known sequence to hybridize with RNA in situ at different tissues. LOSP was located in testis, in round cells located in groups closer to the distal part of the gonadal lobes. The results were published in two papers (Lobov et al. 2015 and 2018).

CHAPTER 5: LOSP polymorphism as a factor of reproductive isolation of molluscs of the genus *Littorina*

LOSP function may be associated with specific binding to a globular protein in the female reproductive system. Disturbance of such interaction could be associated prezygotic reproductive isolation. Potentially it could be involved in other functions as well (blockage of the female seminal receptacle after copulation, agglutination (and survival) of eusperm, etc. An indirect way to check the functional relevance of any gene is to check the ratio of synonymous and non-synonymous substitutions (dN/dS), which can be used to suggest positive selection and therefore confirm pattern of important functionally associated to within/between species scale. They observe different patterns of the evolution ratio in different groups for LOSP: for the obtusata-fabalis group they found a negative ratio suggesting selection cleaning the populations from deleterious alleles. On the other hand in the saxatilis group they typically observed positive ratios, which suggest that positive selection is presently working on the gene. At the same time they found larger level of intraspecific polymorphism and lower level of interspecific polymorphism in the saxatilis compared to the obtuse-fabalis groups. They interpret these patterns as if the postcopulatory effects were more important in the saxatilis group compared to the obtusata-fabalis one. I am not sure of this interpretation and in my

opinion could be still premature, but the pattern observed is interesting and demand of further research in this topic. Apparently this chapter has not yet published.

FINAL ASSESSMENT

This thesis and the published work of the authors that summarised, represents a substantial body of novel and informative work carried out for several years, either alone or in collaboration with colleagues. Through a combination of fieldwork, capture of copulates between species in situ, studying the divergence in RNA and proteins for several tissues and identifying its functional relevance indirectly by measuring the evolution ratio (non synonymous to synonymous substitutions) they made a significant contribution for understanding the evolutionary mechanisms contributing to species identity via reproductive isolation, in particular focusing on postcopulatory mechanisms which has been rarely studied in the past, a contribution that would be considered to meet the criteria for the Degree of Doctor in Biological Sciences at any institution.

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**ROLAN
ALVAREZ
EMILIO - DNI
36061830S**

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Fecha: 2021.01.21 16:03:57 +01'00'

Signed by Emilio Rolán-Alvarez