

THE EVOLUTION OF FLAT PERIWINKLES *LITTORINA FABALIS* AND *L. OBTUSATA* EMPHASIZING MITOCHONDRIAL INTROGRESSION AND RESTRICTED RECOMBINATION

Akademisk avhandling

som för avläggande av doktorexamen i marin ekologi vid institutionen för marin ekologi vid Göteborgs universitet kommer att offentligen försvaras i stora föreläsningssalen, Botanhuset, Carl Skottbergsgatan 22b, Göteborg, fredagen den 3:e oktober 2008 kl. 15.00.

av

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Avhandlingen baseras på följande arbeten:

- I** Kemppainen P., Panova M., Hollander J., Johannesson K. (submitted manuscript). Reticulate evolution in marine snails – Evidence from morphology, microsatellites and mitochondrial DNA
- II** Kemppainen P., Van Nes S., Ceder K., Johannesson K. 2005. Refuge function of marine algae complicates selection in an intertidal snail. *Oecologia* 143: 402–411
- III** Bierne N., Tanguy A., Faure M., Faure B., David E., Boutet I., Boon E., Quere N., Plouviez S., Kemppainen P., Jollivet D., Moraga D., Boudry P., David, P. 2007. Mark–recapture cloning: a straightforward and cost effective cloning method for population genetics of single copy nuclear DNA sequences in diploids. *Molecular Ecology Notes* 7 (4): 562-566
- IV** Kemppainen P., Lindskog T., Johannesson K. (manuscript). A test of the local adaptation mechanism for ecotype linked chromosomal rearrangement in the marine gastropod, *Littorina fabalis*.



UNIVERSITY OF GOTHENBURG

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ABSTRACT: The evolution of species takes in general very long time and different mechanisms are likely to operate during the various stages of this process. Accordingly speciation should be studied at different levels of species divergence. In this thesis I have studied ecological and genetical differentiation between two ecotypes of *Littorina fabalis* as well as between *L. fabalis* and *L. obtusata* - two closely related, directly developing, marine, intertidal gastropods. In *L. fabalis* size is about 25% larger in moderately exposed habitats compared to sheltered habitats and in this thesis I present data showing that this genetically inherited size difference is maintained by an interaction of several selective forces, including life history optimisation, size selective crab predation, fucoid algae functioning as refuges from crab predation and wave-induced dislodgement.

The two ecotypes of *L. fabalis* differ also in the protein arginine kinase (*Ark*) and a Randomly Amplified Polymorphic DNA (RAPD) locus and this linkage disequilibrium persists in locations where both ecotypes are present suggesting that recombination is strongly suppressed between *Ark*, the RAPD locus and one or several loci influencing size. Chromosomal rearrangement, in particular inversions are very effective in restricting recombination and if locally adapted alleles in at least two loci on the same chromosome occur in heterogeneous environments, an inversion may immediately protect these from being mixed up with alleles (introduced by migration) that are locally adapted for other microhabitats. This model predicts that differential selection on these alleles exist before an inversion appears and I have tested this by sequencing an intron of *Ark*. The SS-ecotype was nearly fixed for one haplotype while the diversity among LM-ecotypes was much higher supporting a scenario where a recently derived inversion (or other kind of chromosomal rearrangement) restricts recombination between *Ark* and one or several loci that influence size. In this thesis a novel method used for the sequencing of the *Ark* intron that does not require the cloning of each sample individually (which is both time consuming and expensive) is also presented.

Littorina fabalis and *L. obtusata* are considered as well defined species with clear differences in ecology, morphology and nuclear DNA (allozymes) and with microsatellites I could show that hybridisation between these species has not been occurring at least during the last 10,000 years (they are easily identified in the field by both size and coloration). Despite this they show no consistent differences in the mitochondrial *cyt-b* gene, which could either be due to incomplete lineage sorting or introgression. The idea that mitochondrial DNA can be used as a barcode in species identification is attractive but has in recent years gained criticism because the nature of the mitochondrial molecule makes it specifically prone for introgression between species. Locally restricted mitochondrial introgressions are common among closely related species but the flat periwinkle case study in this thesis clearly shows that a lack of mitochondrial divergence can also exist throughout the whole distribution range for a geographically wide spread species (*L. fabalis* and *L. obtusata* occur sympatrically from Spain to Iceland and the White Sea, Russia).

Keywords: Local adaptations, chromosomal rearrangements, restricted recombination, speciation, mitochondrial introgression