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Evaluation of the habilitation thesis of Clément Lafon-Placette

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Dear Committee members:

I do not know Dr. Lafon-Placette personally, and have not met him, as his research area is rather different from mine (I mainly work on sex chromosome evolution, and for the past 5 years have concentrated on a fish system, not plants). I have, however, worked on population genetics of species in the plant family Brassicaceae, and am aware of research on these species, and some of the interesting questions being studied. I feel that I am able to evaluate his thesis. The guiding question of the submitted thesis is: Do sexual selection and sexual conflict play important roles in the plant speciation and the evolution of plant reproductive systems? My expertise is probably greatest for the topics of the transition from outcrossing to selfing, and sexual selection in plants.

These are important areas of evolutionary biology, and the applicant has participated in studies concerning mechanisms involved in plant speciation, and these have been published in good quality journals. One set of studies has investigated seed inviability of hybrid plants and the involvement of poor endosperm function due to dosage imbalance between alleles contributed by the paternal and maternal plants. This idea is supported by observations of reciprocal differences, though such differences could arise in several ways. The involvement of the endosperm has been studied in Prof. Köhler's group, an excellent lab, in which the applicant trained, and paper III showed that imprinted genes are preferentially deregulated in hybrid seeds between *Capsella* species, suggesting that (unlike in *Mimulus* hybrids, where cyto-nuclear incompatibility is often important, and Kerwin and Sweigart recently showed that mis-expression is a consequence of hybrid sterility), regulatory divergence may be important in *Capsella*, possibly by affecting genes regulated by imprinted genes.

It is important for someone working on speciation to have the expertise needed to genetically map incompatibility loci, as has been very successful in *Mimulus*. The applicant's paper VIII includes such a study, in *Capsella*, which detected three factors, all of which are in the gene-poor, repeat-rich pericentromeric low-recombination regions. I would question the the conclusion that noncoding sequences may largely cause hybrid seed incompatibility, as genes in these regions may still be important, though small RNAs could, of course, be important, as well as imprinted genes, whose epigenetic regulation may often involve these RNAs. I feel, however, that one cannot yet see evidence suggesting that transposable elements play a major role in such evolution.

Given that the mechanistic basis for hybrid seed inviability remains unclear, the candidate's

work also includes research on factors that may lead to the underlying differences. Specifically, the thesis outlines how the transition from outcrossing to selfing modifies the selective processes acting on sexual reproduction, and discusses whether conflict between pollen and ovule genomes is involved, and may cause “arms race” situations. The “Weak Inbreeder/Strong Outbreeder” hypothesis proposes that conflicts between male and female functions are reduced in inbreeders, so that parent-specific selection in endosperm is less likely to be important than in outcrossers. Hybrid seed lethality associated with differences in something called the EBN number (which is not explained, but estimates, or perhaps expresses, endosperm strength in some way), with selfers having the predicted lower values than outcrossing species, and values of more recently evolved selfers differing least from those of closely related outcrossing species (though a caveat is that the number of comparisons is currently too small to show a statistically significant effect).

However, hybrid seed lethality and EBN differences are also found between outcrossers, and paper I proposes that a relationship might exist between genetic diversity and the efficacy of parental conflict, because higher diversity increases either competition during male reproduction, or (through its correlation with effective population size) the efficacy of selection. A colleague at Umea University, J Leppälä, proposed that these ideas can potentially be tested empirically, as clonal reproduction also reduces diversity, and indeed obligate outcrossers with poor clonality prove to have higher EBN index values than ones that can also reproduce clonally, while selfing species have the lowest values of all (though it seems that only one such species was included in the analysis).

In addition, post-zygotic reproductive barriers are likely. One interesting line of enquiry concerns the old observation that polyploidy may allow hybridization in one direction, maybe by raising EBN values above a threshold that would otherwise prevent this. New work in which the applicant took part (Paper IV) suggested that this can allow hybridization in nature.

The candidate’s work on pre- and post-sexual selection in plants, including the extent to which pollen competition involves interactions with the female partner. Again, selfers might be predicted to have less strongly competitive pollen, and indeed a student found higher pollen germination rates in outcrossing than selfing races of *A. lyrata*, and a similar difference between *A. arenosa* and *A. thaliana*, but no differences in pollen tube growth. The finding that female flowers have smaller petals hermaphrodite ones in a gynodioecious, plant may help study the effect of sexual selection. However, a caveat is that such differences may not result from selection, but from developmental constraints involving anther-petal homology. The expectation that differentially expressed genes between in selfing and outcrossing *A. lyrata* may reflect effects of sexual selection may also be questioned, as many functions may differ in the two sexes. Transcriptome studies may thus not prove illuminating.

Overall, there are many interesting questions for study. The applicant has gained expertise in relevant methods for studying these questions empirically and has contributed to many good quality studies. His commitment to research is clearly strong, and, his training experiences have been excellent. Despite my caveats expressed above about a few individual conclusions, my judgement is that he promises to have a good future in the field, and is qualified to train students.

Yours sincerely,

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