Universidad Rey Juan Carlos

- Ph.D. Thesis candidate: *Ondra Hornych*

- Title: *Reproduction and hybridization in ferns*

- Supervisor: *Dr. Libor Ekrt*

**GENERAL EVALUATION**

The thesis is original and ambitious, well planned and well resolved. The text is clear, the data are well analyzed statistically, and the figures and photographs are timely and of high quality I think that the main merits of each chapter are:

*Ch. 1, introduction.* Simple and clear introduction to the main aspects of the life cycle of ferns. It is a useful review for the non-specialist reader to understand the chapters that follow. The candidate demonstrates a good knowledge of the current and older literature.

*Ch. 2, spore abortion.* The most complete study to date on fern spore abortions, with an impressive dataset of 23 individuals, 109 individuals, and 1000 spores per individual. Variation between taxa, ploidy level, genus, reproductive system, and part of the frond is assessed.

*Ch. 3, antheridiogens.* This chapter includes both an experiment with an exceptional number of species (68) and a complete review and meta-analysis of everything published on antheridiogen pheromones. The candidate brilliantly discusses the results and even proposes new hypotheses on the role of antheriogens.

*Ch. 4, hybridization.* The first quantitative assessment of hybridization in ferns, based on a large sample of populations distributed in several European countries. The choice of three species with different degrees of genomic similarity is successful, since the results are very different depending on the pair of species considered.

All chapters are published in the highest quality botanical journals, which is not easy when working on ferns, a small group of plants to which few researchers are dedicated. The candidate demonstrates the ability to lead novel and relevant research and thus the thesis fulfills the requirements for awarding the PhD degree.

**QUESTIONS**:

**1)** *Ch. 2, spore abortion.* The marginally differences between *Asplenium* and *Dryopteris* sexual species is an unexpected result that is difficult to explain. In the Discussion (p. 36), the candidate suggests that could reflect 'different habitat preferences or different position of phylogeny tree', without further detail. In the Fig. 3 (p. 32) I see that variances of each genus are very different, bigger for *Dryopteris*. In addition, this genus shows two outliers far from the median. Are these specimens true outliers that can be removed from the analysis? If so, I think that the differences between genus would be non-significant.

**2)** *Ch. 2, spore abortion*. To me another unexpected result is the different percentages of abortion between parts of the same frond (p. 31). The candidate recommends sampling 'at least two distinct parts of the frond' (p. 37). Is there any biological explanation that can explain this position effect?

**3)** *Ch. 3, antheridiogens*. Willson (1981, DOI: 10.1016/0022-5193(81)90112-0) proposed that antheridiogens are allelopaths secreted by females to stunt the growth of unrelated individuals. The candidate suggests this hypothesis for apomictic ferns (p. 72-73) but not for sexual species. Given the low specificity of antheridiogens, I believe that this role of reducing competition may be as important as that of favoring outcrossing between gametophytes. In fact, the great ecological success of *Pteridium* may be due in part to its ability to inhibit the growth and archegonia formation in gametophytes of many species (as showed in Figure 8b, p. 67). From the 498 pairings (supporting information Table S3, p. 93), the proportions of 'Yes' responses could be compared between pairs with a conspecific and pairs with another taxon, for example with a chi-square test. These simple calculations would serve to quantify the degree of nonspecificity of the antheridiogen effects.

**4)** *Ch. 4, hybridization*. The null model of completely random mating is similar to the minority exclusion model proposed by Levin (1975, DOI: 10.2307/1218997). This model also predicts that the frequency of a given hybrid is $2pq$, where $p$ an $q$ are the frequencies (proportions) of the parent species. However, the corresponding curve (dashed line of Fig. 5, p. 122) does not conform to this formula arcsine transformed or not transformed. For example, if p = 0.5,

$2pq = 0.5$ . . . . (not transformed)

arcsine $(\sqrt{(2pq)}) = 0.79$ . . . . (radians)

$57.3 \times$ arcsine $(\sqrt{(2pq)}) = 45.0$ . . . . (degrees)

whereas in the figure, the y-value is ca. 0.85.

How this curve was calculated?

The data in this chapter are of exceptional quality and quantity to test the Levin's model. I think that frequencies of hybrids equal to or exceeding $2pq$ are strong evidence that there are no (or very weak) reproductive barriers between the parental species.

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