

Referee report on the PhD thesis

Evolution of the genus *Arabidopsis* in its centre of diversity

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The present thesis deals with biogeography and evolution of two wide-spread taxa of the family Brassicaceae applying a number of methods including cytogenetics, genetics, genomics, morphology, and various modelling approaches including ecological data. The comprehensive sampling of the two taxa, diploid *Arabidopsis halleri* and the diploid as well as tetraploid species complex of *Arabidopsis arenosa* allowed addressing a number of issues to resolve the puzzle around these two species, each afflicted with a number of taxonomic uncertainties.

In detail the study of cytogeography using FCM and chromosome counts in the *Arabidopsis arenosa* group revealed a pattern of diploids occurring in southern latitudes (with few exceptions) and tetraploids mostly in northern latitudes. Contact zones of the cytotypes were identified in the Western Carpathians and Slovenian For-Alps. From studying niche differentiation between the cytotypes the authors concluded that random effects such as drift, colonization history play a more important role in cytotype distribution than ecological factors.

In a following case study genome-wide SNPs and microsatellites, habitat data and bioclimatic variables were used to assess genetic structure and niche differentiation in diploid *Arabidopsis arenosa*. The finding that most of the genetic diversity was found North of the Pannonian Basin and in the Carpathians point to these regions as glacial refugia. Ecological factors do play a role in the genomic/genetic structure although niche differentiation was found mostly along gradients related to altitudinal bioclimatic changes. Admixture between the two Carpathian groups might have been a favourable pre-condition for colonizing the isolated Baltic coast. Finally, this is another example, that the genetic structure revealed in this study disagrees with current taxonomic concepts. Obviously, there is a need of dealing with this problem.

Case study 5 comes back to both cytotypes of *Arabidopsis arenosa* in their entire European distribution range asking whether niche shift between cytotypes could be detected if genetically defined tetraploid groups are compared with diploid ancestors. For this additional genomic data of the tetraploids were analysed to resolve the genetic structure within them. Using climatic niche data it turned out that all possible outcomes (niche conservatism/contraction/expansion) could be observed between tetraploid groups and their single diploid original group.

Two further case studies (3, 4) deal with diploid *Arabidopsis halleri*. AFLP data revealed three solid groups in Europe. Divergence estimates based on sequence data pointed to a Pleistocene split. High diversity and rarity indices in certain regions indicate glacial survival in refugia of eastern Central Europe. Another finding namely that morphological variation is essentially determined by environment complicates again the taxonomic treatment of the genetically different groups. Such a taxonomic reassessment was attempted in a following study using molecular and morphological results. Based on this the authors propose a new intraspecific classification, which in fact is discussed but no formal taxonomic summary is presented.

Regarding more formal aspects the language and writing is adequate and the way how the results are presented and discussed is well done. A minor item to reconsider in future papers: While in most figures the inserts showing details are very helpful there are few (e.g., in tree inserts in Fig. 4 of paper 3; in Fig. 3 of paper 4) with details not easy to read. Also the labelling in Fig 2 of paper 5 is challenging. So it is hard to extract detailed information because of too small letters. Concerning bias or conflicts of interest in the presented work I do not see any problems.

Of course in such a complex endeavour always some questions remain:

- (1) Concerns reproduction in general: *Arabidopsis halleri* is a clonal, self-incompatible and highly outcrossing perennial – while *A. arenosa* shows different levels of selfing vs outcrossing.
 - Are there specific pollinators?
 - Regarding the mixed mating system in *A. arenosa*: any indication for specific groups/specific habitats/regions correlated with higher selfing?
- (2) Concerns case study 4: Following the statement (p895): "We ... present here a new infraspecific classification of *A. halleri* based primarily on genetic and geographical patterns..." there is some discussion on the changing circumscription that would require a short taxonomic summary, which I miss here as well as a determination key despite the problems of intersecting morphology.
 - An essential question here is: Can genetics and allopatric distribution be sufficient to define a subspecies?
- (3) Concerns case study 3: Japanese populations of *A. halleri* neither result from old vicariance nor from recent (anthropogenous) spread. You propose LDD probably during Pleistocene. On the other hand the seeds of these taxa are not well adapted for LDD.
 - How could they have made their way?
 - Can we be sure that there are no stepping stone habitats/populations somewhere in Asia between Europe and Japan?

Final evaluation:

Generally speaking this is an excellent investigation on a widespread group of non-model plants of the colline to montane or subalpine altitudinal belt. Such investigations using state of the art methods on sufficiently large samples are in fact rare and, therefore, I consider the papers presented here as an important contribution for a better understanding of the evolutionary history in those plants. The contributions of the candidate to the single papers are significant and the general chapters of the thesis contain an up-to-date survey on biodiversity hotspots in Europe and some considerations on speciation and taxonomy. Furthermore the general chapters provide a good and extensive synthesis of the case studies involved in this thesis.

Considering these facts I recommend this thesis for the defence!

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Ao. Univ.-Prof. i.R./Dr. Josef Greimler

