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Subject matter: Review of PhD thesis of Hana Daneck-Dvořáková

Dear commission,

I have carefully gone over the thesis presented by Hana Daneck-Dvořáková. The thesis presents comparative phylogeographic studies in the region of Central Europe using plants as model systems. Few phylogeographic studies of taxa with this kind of distribution have been carried out up to now and these were mostly in tree species. Thus, the present thesis adds important information to our understanding not only of individual species histories, but also of vegetation changes in Central Europe during and since the last glaciation in general. One of the main questions asked by the three individual studies is which role the Central European region played as a potential Pleistocene refugium for Central European forest species.

The individual studies have been carried out using state-of-the-art methodologies and the methods seem to have been carried out carefully. It is a plus of the individual studies that the plant taxa have been sampled extensively throughout their present distributional area. Two of the three case studies have already been published, i.e. they were critically reviewed, another plus of the PhD thesis.

The calcicole *Hordelymus europaeus* is a grass forming tussocks in beech forests. Indeed, large portions of its distributional range have been sampled, from the Cantabrian Mountains in Spain in the West to the Greater Caucasus in Russia in the East. The results suggest partly congruent and partly incongruent Pleistocene refugia for *Hordelymus europaeus* and the dominant forest tree species *Fagus sylvatica*, suggesting partly concordant migratory movements of these ecologically tightly linked species.

The ecology of *Lonicera nigra* is similar to that of *Hordelymus europaeus*. This shrub is also confined to montane beech forests as well as to mixed forests of spruce, fir and beech. It is confined to basic soils and reaches the subalpine altitudinal belt. Again, the sampling is rather extensive across the distributional area of the species. The results indicate the possibility of a Central European refugium.

The ecology of *Rosa pendulina* is very similar to that of *Lonicera nigra* as well. This shrub grows in the montane to subalpine altitudinal belt, in deciduous and mixed forests as well as knee pine shrubbery, and prefers basic soils. The species has been sampled extensively throughout its distributional range, from the Cantabrian Mountains in Spain in the West to the Rila Mountains in Bulgaria in the East. The phylogeographic pattern revealed by AFLPs in *Rosa pendulina* is highly concordant with that in *Lonicera nigra*, suggesting a common Pleistocene history of these ecologically linked species.

The molecular markers used are appropriate for the questions being asked. Chloroplast DNA intron sequences have been used for *Hordelymus europaeus* and *Lonicera nigra*. AFLPs have been employed for *Lonicera nigra* and *Rosa pendulina*. However, the AFLP data of *Rosa pendulina* complement chloroplast DNA data gathered in a previous study. Thus, in two species – *Lonicera nigra* and *Rosa pendulina* – the two complementary markers have been used, whereas in one species, *Hordelymus europaeus*, only one marker (cpDNA) has been used, which might limit the conclusions that can possibly be drawn from the data. However, variation was recovered with cpDNA data in *Hordelymus europaeus*, which justifies the use of just this single marker system.

As a last point, I have to mention that the English language of the thesis is still a bit poor in some instances – a point, which has of course nothing to do with the scientific quality of the work presented. However, the defendant should consider improving her English skills as this is the main scientific language worldwide.

In summary, I would like to say that this PhD thesis treats an important subject – comparative phylogeography of Central European herbs and shrubs confined to forests –, that it has been carried out in a state-of-the-art manner, and that the results have been presented very well. The results are interesting and suggest the existence of common histories of Central European forest species. It is definitively a felicitous thesis.

Yours sincerely,



Karin Tremetsberger

#### Questions:

1. Based on your knowledge of species ecologies and characteristics, what was your reason for choosing exactly these three species as model organisms for answering your questions?
2. Is anything known about ploidy level variation in your model species? How could ploidy level variation interact with Pleistocene climatic fluctuations?
3. What was the reason for choosing the two different molecular markers, chloroplast DNA sequences and AFLP? What are the properties of these markers and what is the added value of using both of them? In one species, *Hordelymus europaeus*, you employed just one of the markers; would you expect different results/interpretation of data if you had used the other marker as well (similarly to *Rosa pendulina*)?
4. In one species, *Lonicera nigra*, fossil pollen grains have been found. What do you know about the larger phylogenetic and biogeographic context of your species – How old might they be? Where might they have originated?

5. You relate genetic diversity in your study species to the last glaciation in Europe. How do you know that it was the last glaciation that shaped genetic diversity in the species rather than older or younger earth history events?
6. More generally, what are the major achievements that phylogeography has made in the last 2 decades? What are the connections of phylogeography to population genetics, phylogenetics and biogeography (see Avise 2009)?
7. Do you assume that your molecular markers are neutral or that they are under directional or balancing selection? What implications does neutrality or selection have for your interpretation of the data?
8. Based on your knowledge of species ecologies, how would you explain the lack of indications for glacial survival in Central Europe for *Hordelymus europaeus*, but the presence of indications for glacial survival in Central Europe for *Lonicera nigra* and *Rosa pendulina*?
9. Outlook: How could you carry on your research from where you have reached? Which open questions remain?

More specific questions regarding the molecular markers used:

10. Regarding the chloroplast DNA sequences, did you encounter problems in the alignment, e.g., gaps or single nucleotide stretches? If yes, how did you treat these problems?
11. *Lonicera nigra* and *Rosa pendulina* – calculation of DW based on AFLPs: The populations sampled are distributed unequally in the different regions. For example, the sampling of *Lonicera nigra* in the Czech Republic is very dense, whereas it is sparser in the other regions. Could this sampling scheme have affected the inferred values of DW? Similarly, the number of individuals in each population sampled is also not equal. Again, how could this have affected the inferred values of DW?
12. The number of individuals analyzed per population is very low: the minimum is one single individual in some populations. Do you think that this might influence the results of your molecular analyses?
13. In AFLPs of *Lonicera nigra*, you found a high proportion of monomorphic bands (41%). How could this be explained? The reproductive systems of plants are known to highly influence levels of genetic variation within and among populations. What is known about the reproductive system of *Lonicera nigra*? According to East E. M. (1940: The distribution of self-sterility in the flowering plants. Proceedings of the American Philosophical Society 82:449-518) it is a self-compatible species and should therefore have a mixed mating system, which would be consistent with the AFLP results.