Assessment of the PhD-thesis of Jana Raabova, entitled "Local adaptation of the rare herb *Aster amellus* in fragmented landscape"

Jana Raabova has in the work for her thesis studied local adaptation and potential outbreeding depression resulting from crosses between populations, using *Aster amellus* as a model species. More knowledge of the scale and strength of local adaptation is very important for conservation and the results of the thesis contribute to ecology in general and are also of practical value.

The thesis consists of a General Introduction and three chapters that are written as manuscripts and have each their separate introduction, methods and results parts, and a discussion. In the General Introduction Ms Raabova outlines the problems facing plant populations in today's fragmented landscapes, explaines why a better knowledge of the extent of local adaptation and of outbreeding depression is important for conservation, and develops the questions she tries to answer in her thesis. Finally, the biology of the model species *Aster amellus* is presented. The background given is sufficient and well presented, and the theme of the thesis is developed logically from the state of science. The choice of *A. amellus* as a model species appears to be a good one, because it is a species whose populations are fragmented, but is not yet too rare for a large study, and may be representative for a larger number of species. Moreover, two cytotypes can be compared in the Czech Republic, which is an interesting aspect.

Using reciprocal transplant techniques, Ms Raabova could show in Chapter 1 that there is niche differentiation between the two cytotypes as well as local adaptation to the conditions at the sites where the individual populations are growing. There appears to be little gene flow between populations of the two cytotypes, which may have developed in different areas. This chapter nicely demonstrates local adaptation at a rather small scale and adds to the literature on niche differentiation between cytotypes. Chapter 2 investigates the importance of ecological vs. geographical distance for local adaptation in *A. amellus* and shows that differences in ecological conditions explained more of the performance differences among plants than geographical distance. This is an interesting result and an important contribution, because there are few studies that have compared the two factors in a meaningful way. Chapter 3 compares the fitness of the F1-offspring from crosses within populations, between populations within regions and between populations from different regions to assess the potential importance of outbreeding depression. No outbreeding depression was found in the F1-generation. A strength of the study is that the fitness of different offspring was not only studied in a common garden, but also in the field. A certain weakness is that only the F1 was studied, but this is freely admitted and has been the case in nearly all similar studies. The

results of the study add to the growing body of evidence that potential outbreeding effects may not be very important in conservation planning.

The studies and experiments are well designed. Ms Rabova has employed and mastered for her thesis work an impressive number of techniques and methods, ranging from molecular genetic analyses to common garden and field experiments, pollination experiments, and vegetation studies. The thesis is generally well written, logically constructed and there are very few problems with the English. Figures and tables nicely illustrate the results, although occasionally they do not show everything that is stated in the text. The conclusions are valid and do not exceed the results.

Most of my criticisms concern the statistical analysis. The analysis of reciprocal transplant experiments is very complicated and there are some open questions concerning the statistical analyses of the data in the thesis which should be discussed during the defence (see detailed remarks).

One of the chapters has already been published in the journal "Biological Conservation", showing that it is highly valued. Overall, I consider the thesis suitable for the defense and I think it fulfills the criteria necessary for a PhD-degree.

Prof. Dr. Diethart Matthies

Questions to the defendant

- Why was genetic distance between populations, based on the analysis of neutral genetic markers (isozymes), included as a possible explanation for differences in the performance of plants at different sites (study 2)? You state that divergence in molecular markers may indicate ecotypic variation (p. 58). Could you explain the factors responsible for divergence in supposedly neutral markers and those responsible for ecotypic differentiation? What is known about the correlation between measures of genetic differentiation based on neutral markers and those based on quantitative traits that are under selection?

- The size of the populations in study 2 varied from 60 - 10 000 individuals. What effects could population sizes have on the strength of local adaptation?

- At only two sites had local plants a higher flowering probability than foreign plants (study 2), indicating local adaptation. Why may flowering percentage of transplants have increased at most sites with increasing differences in local climate between sites (p. 54)? You state (p. 65) that ecological differences tend to be independent of geographical distance. Would you therefore expect no relationship between differences in environmental conditions and geographical distance between sites? What is known from other studies about the effects of geographical distance on adaptation?

- Could you explain the general principles of the analysis of data from nested designs and why you have chosen the residual as an error term for testing the effects of target site and population? What was the error term for the effect of region? How were standard errors for the effects of region, population etc. calculated?

- Could you explain the potential practical importance of the results of your studies for conservation? What further studies would be necessary?

Specific comments

Chapter 1

- I wonder what the difference between question 1 and 2 is (p. 21).

- I wonder how it is possible that there are no differences among sites in pH, but strong differences in carbonate content, which is usually strongly correlated with pH (Table 1).

- It is stated that the effects of target site, population of origin and their interaction was tested against the residual (p. 26). This ignores the nested design of the study (plots nested within sites in the sowing experiment and rows within sites in the transplant experiment) and inflates the statistical power of the analysis. Unfortunately, it is not possible to check the error terms used in the analyses, because only F and p-values, but not mean squares or mean deviances are given in the statistical tables.

- In Table 2 and 3 values for the R^2 of various effects are given. These values are mostly larger 1 and I assume that they are therefore not R^2 - values (which should be < 1), but the proportion of variation due to the respective factors. However, I think that the calculation of these values in a nested analysis of variance (Table 2 and 3) is not appropriate, because they do not add up to 100%, since there are several error terms in the model.

- On p. 30 it is stated with reference to Fig. 2c,d that plants had larger leaves at sites with high than low productivity. This may well be so, but Fig. 2 does not show this. Instead it shows differences between ploidy levels.

- Similarly, Fig. 4c which is referred to on p. 31, does not show that flowering percentage was higher at sites with high than low productivity.

- Error bars in Fig. 2d, and Fig. 4b are suspiciously small. Moreover, the number of plants is given as the number of replicates on which the standard errors are based. This ignores the nested design of the study. Replicates for the effect of habitat type, for instance, are the populations, not the individual plants.

- Which species is *M. annua* (p. 36)?

Chapter 2

- Data on soil properties were standardized prior to calculating environmental Euclidean distances between sites to eliminate effects of different measurement units (p. 49/50). However, potential solar irradiation and cover of individual plant species are also measured on very different scales. Why were those data not standardized?

- It is stated that the effects of target site, population of origin and their interaction was tested against the residual (p. 51). This ignores the nested design of the study (plots nested within sites in the sowing experiment and rows within sites in the transplant experiment) and inflates the statistical power of the analysis. Unfortunately, it is not possible to check the error terms used in the analyses, because only F and p-values, but not mean squares or mean deviances are given in the statistical tables.

- The proportion of bare soil cover was used as a covariate in the analysis of the sowing experiment and had strong effects on plant performance (Table 4). If sites varied in the proportion of bare soil, this use of a covariate in the analysis may mask effects of target region and population (= site).

- What were the replicates used for the calculation of the standard errors in Fig. 2?

- It is suggested, that differences in bare soil cover might account for the differenecs in germination percentage among sites and be a reason why only some populations showed significant local adaptation (p. 62). However, bare soil cover was used as a covariate in the analyses and its effect thus eliminated.

Chapter 3

- On p. 74 it is stated that in general all types of crosses were performed for each plant. I would therefore expect that the plant individual is included as a block effect in the statistical analysis (Tables 2, 3 and 4).

- To assess the influence of crossing distance on offspring performance, two seeds from each flowerhead were selected and grown in two different substrates in a common garden experiment (see p. 75). The two seeds from an individual flowerhead are not independent samples and this has to be taken into account in the statistical analysis. The flowerheads

represent the replicates for the various effects of crossing distance in the statistical analysis. Currently, the effects of crossing distance are tested against the residual variation among plants which inflates the statistical power of the analysis (Table 3).

- Consequently, the standard errors given in Fig. 2 are likely to be too small, because they are probably based on too large a number of replicates.