Summary

The research carried out within the framework of the presented thesis aimed to better understand the demographic structure of the Greek populations of horse-chestnut (*Aesculus hippocastanum*), as well as to determine their genetic resources and the potential impact of climate change on the species' range. Since a detailed knowledge of natural populations is crucial for planning conservation strategies for the species, the following objectives were set in this study: 1) to analyze the demographic structure of natural populations of horse-chestnut and the degree of their infestation by horse-chestnut leaf miner; 2) to determine the impact of climate change on the species' range; 3) to determine the genetic variability and genetic diversity in natural populations of horse-chestnut and the factors affecting gene flow between them.

Investigated stands were characterized by a diverse demographic structure and a high number of seedlings. The horse-chestnut is distinct compared to many other relict species, which are usually characterized by poor natural regeneration. This means that the hypothesis that insufficient natural regeneration is a factor limiting the spread and replacement of horsechestnut generations should be rejected. Probably more important factors impeding colonization are horse-chestnut biology, competition with other species, and habitat specialization.

The level of infestation by horse-chestnut leaf miner was very diverse and depended on the altitude of the stand. Mines were not found in two natural populations. The factor influencing the low level of infestation of some natural populations is probably their geographical isolation and distance from the road network. The obtained results allowed verifying negatively the hypothesis that natural stands of horse-chestnuts are infested by the horse-chestnut leaf miner to a similar degree as artificial stands.

The results of the theoretical range modelling showed that the horse-chestnut was probably much more widespread in the past, and since the Last Glacial Maximum, its range has significantly decreased. This result supports the hypothesis of the range reduction of the species in the past. The present range is largely influenced by winter moisture conditions, which corresponds to the general adaptation of Mediterranean species. Expected climate change could potentially cause further range reduction but only to a small extent. Only the most pessimistic scenario predicts a threat to natural populations located at the centre of the species' range in the Pindos Mountains. Therefore, the hypothesis that the predicted future climate change will have a significant impact on further reduction of the natural range of the horse-chestnut should be rejected. A much greater threat to the horse-chestnut may be the increasing human pressure, related to the increased consumption of water resources in the Mediterranean region, and the loss of habitat. Other threats are diseases and pests, such as the horse-chestnut leaf miner.

Relict and endemic species often have limited genetic variability due to observed small population size and range fragmentation. These factors are conducive to the loss of genetic variability. Taking into account the features of the occurrence of horse-chestnut, it was hypothesized that natural populations are characterized by low genetic variability and high genetic diversity. However, this hypothesis was verified negatively. The analysed populations

showed moderate diversity and relatively high genetic variability. In the case of endemic woody species, the maintenance of high variability may be related to traits such as longevity and overlapping generations. The fact that the species may have been present in the centre of its current range since at least the last glaciation may also be of great importance.

Historical factors have shaped the contemporary gene pool of many endemic species from the Mediterranean region. The obtained results allowed us to positively verify the hypothesis that biogeographical factors played a decisive role in shaping the pattern of genetic differentiation of the horse-chestnut in its natural range. Habitat characteristics had no statistically significant effect on population differentiation, while the distance between populations was significant.

The level of genetic variation and differentiation between populations is strongly influenced by gene flow. The obtained results indicate that the contact between horse-chestnut populations is difficult, which is mainly related to the complex topography of the Balkan Peninsula. This supports the hypothesis that gene flow between horse-chestnut populations is low.

The species-specific microsatellite markers designed in this thesis showed similar parameters to the cross-amplified markers used previously. The hypothesis that the specific markers show greater polymorphism than the cross-amplified markers should be rejected. The designed markers can be used in the future to determine genetic resources among different taxa of the genus *Aesculus*.

The presented results are an important contribution to a better understanding of the natural populations of horse-chestnut. Good natural regeneration, together with the rather optimistic result of predicting the theoretical range in the future, suggest an important role of in situ conservation in the survival of this endemic species. Conservation of the horse-chestnut could also be enriched by assisted migration, using gene resources from populations with the highest genetic variability. The publications included in the doctoral thesis can provide a theoretical foundation from which an effective conservation strategy can be developed for this species, important for the natural environment of the Pindos Mountains, as well as important for pharmacy and horticulture.