



# Large-scale plant conservation in European semi-natural grasslands: a population genetic perspective

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## ABSTRACT

During the last century, unprecedented landscape fragmentation has severely affected many plant species occurring in once widespread semi-natural grasslands in Europe. Fragmentation reduces population size and increases isolation, which can jeopardize the persistence of populations. Recent large-scale ecological and genetic studies across several European countries indicate that fragmented populations of common plant species exhibit a strong genetic differentiation and local adaptation to their home sites, reducing their capacity to establish new populations elsewhere. We discuss the main genetic processes that determine the performance of plant populations in severely fragmented landscapes: namely inbreeding depression, genetic differentiation and genetic introgression. We stress the need for large-scale genetic studies to detect the geographical structure of genetic variation of fragmented plant populations, since nuclei of genetically independent groups of populations may become important targets of conservation. A thorough knowledge on the large-scale geographical structure of genetic variation for a sufficiently wide array of plant species can provide the basis to develop comprehensive conservation plans to preserve the ecological and evolutionary processes that generate and maintain biodiversity of fragmented semi-natural grasslands.

## Keywords

Distribution range, extinction risk, genetic differentiation, genetic introgression, inbreeding depression, geographical structure of genetic variation, landscape fragmentation, local adaptation, re-introduction.

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## INTRODUCTION

The overall impoverishment of biodiversity resulting from landscape fragmentation seems to be an irremediable threat. To study the implications of fragmentation for species persistence, a multidisciplinary approach is required including the geography, ecology, and genetics of species analysed at large spatiotemporal scales and the development of measures at that scale necessary to recover biodiversity. Large-scale studies are needed because the distribution patterns of several species across their distribution ranges have rapidly changed during the past century. In the case of plants, a few recent large-scale studies at the European continental scale assessed the performance of plant populations based on reciprocal transplant experiments (Joshi *et al.*, 2001; Santamaría *et al.*, 2003; Becker *et al.*, 2006a,b; Bischoff *et al.*, 2006). Some of these studies in particular quantify how large-scale fragmentation acts on the processes of genetic differentiation and the extent of local adaptation of extant populations (see Becker *et al.*, 2006a,b). From a conservation point of view, understanding how these processes are operating at different scales is

extremely important because the success of future restoration actions may strongly depend on the genetic characteristics of remnant populations over vast areas of fragmented landscapes.

Semi-natural grasslands in Central and Northern Europe represent a good example of the scenario described above. Semi-natural grasslands are characterized by their traditional land-use (e.g. mowing and livestock grazing) that exerts a continuous disturbance inhibiting succession and preventing many grasslands specialists from being competitively excluded. As a result, these habitats contain an exceptionally high diversity of plants as well as fungi, insects and birds (Kull & Zobel, 1991; Vessby *et al.*, 2002; Critchley *et al.*, 2003), so they represent important targets for conservation in several European countries. The area occupied by semi-natural grasslands in Europe and the biodiversity they contain have dramatically been reduced during the past century because of a decrease of traditional management (Poschlo *et al.*, 2005; Lindborg, 2006) and an intensification of agriculture (Critchley *et al.*, 2003; Foley *et al.*, 2005). The consequences of this decline are an unprecedented landscape fragmentation and loss of habitat quality

compared to the species' migratory capacity and adaptation potential. As a result, many common plant species in European semi-natural grasslands are now represented by fewer, smaller and more distant populations.

The main consequence of the current situation is that the effects of environmental (i.e. unpredictable effects of environmental factors on demographic attributes) and demographic (i.e. chance events of individual mortality and reproduction) stochasticity on the fate of these fragmented and isolated populations considerably increase their extinction risk (Lande, 1993; Holsinger, 2000; Menges, 2000; Matthies *et al.*, 2004). At the same time, the overall population performance (in terms of recruitment, survival, and fecundity) has clearly diminished. It has been shown that the genetic diversity of extant populations decreases with population size mainly as a result of pollination limitation or genetic drift, and population attributes directly related to population fitness are lower in small populations due to inbreeding and loss of genetic variation (Oostermeijer *et al.*, 1994; Fischer & Matthies, 1998; Kéry *et al.*, 2000; Luijten *et al.*, 2000; Vergeer *et al.*, 2003; Brys *et al.*, 2004; Hensen & Wesche, 2006; Leimu *et al.*, 2006). Overall, demographic and genetic factors seem to act in concert, increasing extinction risk in small fragmented populations (Oostermeijer *et al.*, 2003; Picó, 2004; Frankham, 2005), a situation that certainly applies to plant populations in European semi-natural grasslands.

The eventual genetic effects of fragmentation on population persistence, however, may depend on plant traits, such as longevity, mating system, and dispersal ability. These plant traits modify the relationship between genetic diversity, plant fitness, and population differentiation. In a classical review, Hamrick & Godt (1996) pointed out that woody species had more genetic diversity and less among-population differentiation than herbaceous species, probably as a result of a longer lifespan and greater dispersal distances in the former than in the latter. Mating system also represents a crucial trait to understand patterns of genetic diversity and its effects on plant fitness. In a recent review, a positive association between mean genetic diversity and fitness has been found in self-incompatible but not in self-compatible species (Leimu *et al.*, 2006), which can be the result of the strong dependence of suitable mating partners and a greater susceptibility to small population size in the former than in the latter.

Here we highlight the main genetic aspects that strongly determine the performance of plant populations: inbreeding depression, genetic differentiation, and genetic introgression. We also focus on the consequences that are of interest for conservation biologists and managers of European semi-natural grasslands on the scale of whole regions. We stress the need to understand and forecast the genetic consequences of the progressive landscape fragmentation experienced in ample European regions during the past century, since this knowledge will certainly contribute to better design large-scale conservation plans. Such conservation plans must go beyond the preservation of extant plant populations focusing also on the restoration of depleted populations and/or the creation of new ones in restored suitable sites over the whole distribution area of a species.

## LANDSCAPE FRAGMENTATION AND INBREEDING DEPRESSION

Many plant species in fragmented landscapes occur as small, isolated populations. Population size and degree of isolation in combination with mating system may have a profound effect on reproductive success. Self-incompatible species may exhibit lower fitness values (e.g. fruit set) simply because of a shortage of suitable mating partners (Leimu *et al.*, 2006). In the case of self-compatible and mixed mating species, selfing and biparental inbreeding between individuals significantly increase. The resulting inbred progeny can be characterized by the effects of inbreeding depression (i.e. the reduction in fitness in inbred individuals as compared with their outbred relatives) on several life-cycle traits (Charlesworth & Charlesworth, 1987, 1999; Husband & Schemske, 1996). On top of inbreeding depression, inbred progeny can also exhibit an important loss of genetic variability and the accumulation and fixation of mutations (Dudash & Fenster, 2000 and references therein). Overall, the effects of inbreeding depression on plant fitness traits lead to decreasing population fitness as a whole.

It has been shown that individuals within populations exhibit a great variation in their response to inbreeding depression (Husband & Schemske, 1996; Dudash *et al.*, 1997; Koelewijn, 1998; Mutikainen & Delph, 1998; Ouborg *et al.*, 2000; Rankin *et al.*, 2002; Rao *et al.*, 2002; Picó *et al.*, 2003, 2004; Kelly, 2005). Families within a population respond differently to cross type in a way that some of them exhibit higher fitness under outcrossing whereas others perform much better under selfing. Mixed-mating plants, moreover, exhibit a higher variation among individuals than selfing plants (Parker *et al.*, 1995; Fishman, 2001), a fact that has been attributed to the long history of inbreeding in selfers. Nevertheless, the ultimate causes of such among-individual variation in inbreeding depression are largely speculative, and it has been suggested that individuals might actually differ in the number of deleterious mutations that they carry (Koelewijn, 1998).

Reduction in population size might select for those genotypes exhibiting a higher fitness under selfing. Hence, the proportion of genotypes with a positive response to inbreeding would increase as populations become more inbred, eventually increasing population fitness and reducing extinction risk. Although this hypothetical situation would be ideal to let fragmented populations go without any kind of management (e.g. introduction of reproductive plants), data seem to indicate that such small, inbred populations with high performance do not exist, whereas the negative fitness effects of inbreeding are well documented (Oostermeijer *et al.*, 1994; Fischer & Matthies, 1998; Kéry *et al.*, 2000; Luijten *et al.*, 2000; Vergeer *et al.*, 2003; Brys *et al.*, 2004; Picó *et al.*, 2004; Hensen & Wesche, 2006). We believe that strong demographic stochasticity and the impoverished habitat quality of several small fragmented populations might reinforce this evident fact.

The genetic basis of among-individual variation in inbreeding depression is not completely understood, surely because several processes (e.g. the real extent of purging of deleterious alleles, the

unknown extent of formation and breakdown of co-adapted gene complexes) may act in concert in a complex manner, so predictions on the demographic implications of among-individual variation in inbreeding depression are not straightforward (Keller & Waller, 2002). Hence, advances in the genetic basis of inbreeding depression to better understand and predict the response of different genotypes to inbreeding depression will have important conservation implications beyond the evolution of plant mating systems.

## LANDSCAPE FRAGMENTATION AND GENETIC DIFFERENTIATION

Vucetich & Waite (2003) recently suggested that effective population size (i.e. the number of adults in a population contributing offspring to the next generation;  $N_e$ ) may be a key parameter to understand differences in population performance. They showed that  $N_e$  values strongly determine the rate of genetic drift accounting for the particular genetic characteristics of small populations, such as those near the edge of species distribution or severely fragmented populations. Hence, in the case of plant species from European semi-natural grasslands affected by recent landscape fragmentation, area-related reductions in population size (and therefore  $N_e$ ) and increasing distance between extant populations supposedly will lead to increasing rates of genetic drift across large areas of the species distributions.

Studies of plant species occurring in severely fragmented landscapes indeed clearly show high genetic differentiation between extant populations. This is mainly interpreted as an effect of genetic drift (Lammi *et al.*, 1999; Després *et al.*, 2002; Hooftman *et al.*, 2004; Hensen & Oberprieler, 2005; Mix *et al.*, 2006; Pfeifer & Jetschke, 2006). Intense inbreeding, moreover, increases the rate of genetic drift and subsequently the extent of genetic differentiation among populations. Obviously, the dispersal ability of plant species and the concomitant gene flow between populations will determine the distance above which genetic drift will operate (Mix *et al.*, 2006). Nonetheless, the loss of suitable sites and the habitat deterioration for several common plant species in Europe reduce the extent of seed dispersal and pollination (Steffan-Dewenter & Tschardt, 1999; Willerding & Poschlod, 2002; Honnay *et al.*, 2006) that could counteract genetic drift.

The current situation of pollinators and seed dispersal vectors may also account for the high levels of genetic differentiation in fragmented populations. In vast areas in different European countries, pollinators and pollinator assemblages have been dramatically reduced in just a few decades, and this seems to be casually connected with the generalized decline of insect-pollinated plant populations in the same areas (Biesmeijer *et al.*, 2006; Carvell *et al.*, 2006). The same can be said for pronounced changes in the abundance and composition of seed dispersal vectors (Poschlod & Bonn, 1998; Ozinga *et al.*, 2005; Poschlod *et al.*, 2005; Soons & Ozinga, 2005), especially for those species that depend on water or free ranging animals for their dispersal.

If generation time of plant species is shorter than the history of fragmentation (i.e. a century for many European regions), which applies for several herbaceous plants, then we can expect a strong

genetic differentiation among extant plant populations across large regions of the species distribution area. It must be emphasized that fragmentation would increase the natural level of genetic differentiation and the local adaptation that non-fragmented populations tend to exhibit (see Bischoff *et al.*, 2006). At present, only a few large-scale studies on semi-natural grassland plant species with wide distributions across Europe have analysed the extent of genetic differentiation and local adaptation through common garden and reciprocal transplant experiments using populations from different European regions (Joshi *et al.*, 2001; Becker *et al.*, 2006b; Bischoff *et al.*, 2006). A general result that emerges from common garden experiments is that there exists strong genetic differentiation among populations from different regions. Populations within the same region are genetically more similar than populations of other regions (Becker *et al.*, 2006b). The other general conclusion obtained from replant–transplant experiments is that overall performance of plant species tends to be higher in populations at their home sites when compared with that of populations replanted at away sites (Joshi *et al.*, 2001; Becker *et al.*, 2006b; Bischoff *et al.*, 2006). Furthermore, overall performance shows a clear trend of decline with increasing transplanting distance (Joshi *et al.*, 2001; Becker *et al.*, 2006b).

The management and fragmentation histories of semi-natural grasslands can differ between European countries (i.e. the ‘region’ in reciprocal transplant experiments), which might have affected the current distribution, abundance, and subsequent overall performance of plant populations in each country. Some studies clearly show that historical changes in land use and landscape structure over the past century, as detailed on historical maps, still exert a strong effect on the present-day habitat occupancy and population size of plant species (Donohue *et al.*, 2000; Eriksson *et al.*, 2002; Lindborg & Eriksson, 2004; Herbén *et al.*, 2006; Lindborg, 2006). Although direct empirical proof is lacking, it can be assumed that differences between countries in the fragmentation history of their respective landscapes may also contribute to the observed strong regional-scale genetic differentiation of several plant species.

## LANDSCAPE FRAGMENTATION AND GENETIC INTROGRESSION

The critical genetic situation of many plant species over wide areas in Europe and the limited possibilities to restore the gene flow between them, stresses the need to support weak populations by translocation of seed and/or transplants (van Groenendael *et al.*, 1998; Luijten *et al.*, 2002). Important questions and concerns, however, arise about the quality and provenance of plant material (Hodder & Bullock, 1997; Keller *et al.*, 2000; Visser & Reheul, 2001) to be used to restore species-rich habitats such as semi-natural grasslands. These concerns are totally justified since problems may appear as a result of maladaptation of non-local plants that leads to lower fitness, and genetic introgression of non-local genes that can destabilize the genetic integrity of local populations (Keller *et al.*, 2000), a process known as outbreeding depression. In the case of plant species occurring in semi-natural grasslands, where gene flow between populations is low and

genetic differentiation is high, outbreeding depression is expected to occur which then leads to decreased offspring vigour (Waser & Price, 1994; Becker *et al.*, 2006a).

It is important to quantify the performance of progeny resulting from large-scale crosses between plants from different origins to predict the fitness consequences of restored populations (Dudash & Fenster, 2000; Keller *et al.*, 2000; Becker *et al.*, 2006a). In the case of European semi-natural grasslands, the few studies that conducted large-scale interpopulation crosses concluded that the sensitivity of populations to introgression may widely vary among European regions and that the extent of outbreeding depression does not increase with interpopulation distance (Keller *et al.*, 2000; Becker *et al.*, 2006a). The strong genetic differentiation among extant plant populations in semi-natural grasslands over large areas in Europe could account for the lack of resolution to pinpoint over what distance interpopulation crosses can be conducted to produce high-fitness progeny. Large-scale interpopulation crosses require complicated, time-consuming experimental designs that limit the number of populations within regions that can be tested. However, a general view exists, which is supported by empirical evidence, that plant material for reinforcement of populations should be obtained from local populations or, when possible, from several large populations within the region (Helenum, 1998; van Groenendael *et al.*, 1998; Galloway & Fenster, 2000; Keller *et al.*, 2000; Luijten *et al.*, 2002; Vergeer *et al.*, 2004).

## CONSERVATION IMPLICATIONS

Evidence presented so far shows that plant populations in European semi-natural grasslands exhibit a strong pattern of genetic differentiation and erosion. The conservation implications of the existence of multiple nuclei of isolated and genetically differentiated plant populations over large areas in Europe is that each genetically independent unit becomes an important target for conservation since it may contain genotypes locally adapted to their particular habitat characteristics. At the same time such specialization makes these genotypes unfit for re-colonization elsewhere. As stressed by Hufford & Mazer (2003), there is the urgent need to detect so-called 'seed transfer zones', that is, geographical regions within which individuals of a given species can be transferred with no detrimental effects on population mean fitness. We believe that identifying the geographical structure of genetic variation of widespread plant species in Europe should become a conservation priority because it is conditional to any successful rescue operation.

To achieve this goal, two complementary approaches can be adopted. First, we need to conduct more common garden and replant-transplant experiments using multiple populations over the species distribution range to determine the extent of genetic differentiation and local adaptation in morphological and phenological traits. Second, the genotyping of populations with molecular markers (e.g. microsatellites) and the analyses of genetic variation will allow us to determine the genetically independent groups over the species distribution range by directly analysing the population structure and isolation-by-distance patterns. Overall, results must lead us to recognize characteristics in mating

system, longevity, dispersal ability, dispersal vectors, or habitat preferences already available in large databases (Knevel *et al.*, 2003) by which we can estimate extinction risks and assign conservation priorities. This should give us the potential to design effective conservation actions to protect and reinforce the remaining populations of formerly abundant plant species.

As pointed out by different authors, conservation and restoration genetics should focus on the maintenance of historical processes (e.g. levels of gene flow) across the species distribution range (Spector, 2002; Gao & Zhang, 2005). As stressed by Ennos *et al.* (2005), species-based conservation measures are untenable so it is better to conserve the evolutionary processes that generate biological complexity rather than conserving a limited number of threatened species per se. Understanding the geographical structure of genetic variation of plant species across their distribution range would contribute to understanding the ecological and genetic processes at different scales.

However, there is an important caveat. We have data on how the area occupied by semi-natural grasslands and the occurrence of their plant species have decreased in Europe during the past century. Unfortunately, we do not know the historical processes operating in semi-natural grasslands before the rapid abandonment of traditional agricultural practices and their effects on the geographical structure of genetic variation prior to fragmentation, so direct comparisons are not possible. Nevertheless, it is reasonable to believe that fragmentation tends to increase the geographical structure of genetic variation simply as a direct consequence of increased isolation among extant populations. It must be emphasized that both traditional husbandry and more recent fragmentation were driven by socioeconomic considerations. Hence, the restoration of semi-natural grasslands with their species and processes (e.g. mowing regimes, grazing intensity) will have to be done in accordance with the current socioeconomic conditions in different European countries, but still pursuing to restore and preserve the high biodiversity that has characterized semi-natural grasslands for centuries.

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