Reproductive biology and gene flow in a fragmented landscape

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Abstract

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After a presentation of recent studies on gene flow and on patterns of genetic variation in plants, a brief evaluation is given of the effects of fragmentation of the landscape on the genetic variation in plant species and the consequential effects of inbreeding depression. Data show that reduced fitness in small and isolated populations occurs, owing to reduced reproductive success (lower fruit or seed set) and because of inbreeding. It is also suggested that the still poorly studied mechanism of outbreeding depression may also have negative effects. In particular, outbreeding species that used to be widespread and common, suffer more from fragmentation and isolation than endemic and naturally rare species or predominant selfers. More emphasis should be placed on the conservation of new rare species, because their ecotypic variation and manifold, but often undiscovered local adaptations may get lost, and consequently also their long-term evolutionary perspectives.

Gene flow

Since Ehrlich & Raven (1969) expressed their doubt regarding the importance of gene flow for the cohesion of species and for the evolutionary perspectives of taxa, much debate on this topic has arisen. The behaviour of foraging animals, the dispersal of pollen and seeds by wind and animals, and the isolating distances kept by seed growers also led Levin & Kerster (1974) to conclude that gene flow must be limited, and could not account for the long distance genetic coherence of plant species. Levin (1979) stated that many of the research data point to a limited amount of gene flow, which made him pose the question whether a plant species constitutes a coherent unit of evolution or just comprises a group of separate subunits sharing few genetic and ecological characters (as for example found in *Triticum turgidum* by Nevo & al. 1988, Nevo & Beiles 1989).

Recent studies report very limited gene flow distances indeed in several species: only approximately 1.5 metres for *Plantago* (Bos & al. 1986, Tonsor 1985), and approximately 14 metres for *Lathyrus* (Godt & Hamrick 1993), Vogel & Westerkamp (1991) also assume only little pollen flow, judging from data showing poor pollen carry-over by pollinators and the generally restricted neighbourhood size, particularly in entomophilous herbs.

However, from other studies it became increasingly clear that many of the gene dispersal experiments (in particular those based on pollen flow) tend to underestimate the real level of gene flow, and that its actual range may be quite larger than hitherto assumed (Schaal 1980, Levin 1981, Friedman & Adams 1985, Ellstrand & Marshall 1985, Ellstrand 1988, Ellstrand & al. 1989, Devlin & Ellstrand 1990, Campbell 1991, Stanton & al. 1992, Ellstrand 1992). We refer to Hamrick, Godt & Sherman-Broyles (1995) for an extensive review of recent gene flow studies.

Pollen movement by itself not always guarantees the actual establishment of the genes dispersed, as has been demonstrated by Rasmussen & Brødsgard (1992) who found no effects of pollen transfer on the genetic structure of a receiving population of *Lotus corniculatus*.

In general, it is important to realize that the majority of the studies mentioned above report on *intra*-populational gene flow. To our knowledge, Ellstrand & Marshall (1985) were the first who explicitly studied *between*-populational gene flow. Klinger & al (1991, 1992) performed studies on pollen flux in *Raphanus*, and found that particularly small populations (in the neighbourhood of large ones) may undergo high levels of gene influx. Ellstrand & Elam (1993) consider this phenomenon in view of the conservation perspectives of small populations of rare species.

There are two indirect methods currently used to calculate the level of gene flow among populations. One is based on the occurrence of private alleles, the other uses all available polymorphisms from allozyme studies (for an evaluation, see Hamrick & al. 1995). According to these authors, the most reliable is the second method. The following data cited are of the latter nature [*Nm(W)*]. There is a strong relationship between the level of gene flow and the breeding system of the species. Hamrick & al. (1995) show that *Nm* values (defined as number of immigrants per generation) may get as high as 5.73 in wind pollinated (and self-incompatible) species. The mean *Nm* value in selfing species is 0.57, with individual species exhibiting values as low as 0.065 and 0.24 (Hamrick 1987, Govindaraju 1988). The animal pollinated and self-compatible wind pollinated taxa show intermediate values of 1.61-2.33 and 1.53, respectively. However, data may be surprising, as for example in the assumingly well-dispersing genus *Taraxacum*, where sexual representatives of the section *Ruderalia* apparently have very limited interpopulational gene flow, as the degree of between population differentiation is quite high (Menken & al. 1995).

Hamrick & al. (1995) state that the mode of pollination and dispersal, the longevity of the individuals, and the size of the population all affect the rate of gene flow. The more gene flow capacity is limited, the more the between-population differentiation will increase (Wright 1965).

It may be evident that a general statement on the level and the range of gene flow is impossible to make. However, in the following review we will see that not only the pollination mode, but also various other aspects of the reproductive biology, life history and historical development of taxa are of major importance in affecting gene flow. This not only is true for the level of gene flow as such, but also for the effects of fragmentation and isolation.

Patterns of genetic variation

In a series of reviews by Hamrick (1983, 1987), Hamrick & Godt (1989), Hamrick & al. (1991, 1995), and Karron (1991), the patterns of genetic variation (based mainly on allozyme markers) has been discussed. It is obvious that some general trends exist.

(1) Species that are endemic or otherwise have a naturally limited distribution have lower overall levels of variation than wide-ranging taxa. An explanation for this may be found in the history of continuity of the population complexes of wide-ranging species, which prevents the loss of variation by genetic drift. In contrast, endemics may have experienced bottlenecks with a subsequent loss of variation (and adaptation to ecologically restricted situations). In rare species the between-population differentiation may be high. A clear example of differentiation between a wide ranging and a narrowly distributed species is given by Cole & Biesboer (1992) for *Lespedeza leptostachya* (rare) and *L. capitata* (wide-spread).

(2) A second important trend is found when different breeding systems are compared. On the species level, selfers have significantly smaller proportions of polymorphic loci and gene diversity. The same holds for the within-population variation, whereas the between-population differentiation generally is stronger. The pattern in outbreeders is largely the opposite: a high level of genetic variation, and relatively little between-population differentiation (Hamrick & al. 1995).

The absence of inbreeding depression in naturally inbreeding species is attributed to the process of purging (Lande & Schemske 1985, Schemske & Lande 1985). This elimination of deleterious alleles in a situation of repeated inbreeding is assumed to reduce inbreeding depression. In *Clarkia temblorensis*, the level of inbreeding depression is lower in populations that are already inbred: the plants have become 'adapted' in some way to selfing (Holtsford & Ellstrand 1990). A similar pattern has been found in *Eichornia paniculata* (Barrett & Kohn 1991).

However, modelling studies show that purging will take many (about 20 to 25) generations of inbreeding (van Treuren 1993). It is questionable whether the present environmental situation in many parts of the world, with the enormous rate of habitat destruction and loss of habitat quality, will give (threatened) taxa, especially long-lived ones, enough time to go through this process (Karron 1991). Furthermore, the type of rarity of a given taxon will be of crucial importance (Rabinowitz 1981). In particular, taxa that were recently decimated and fragmented, the so called 'new rares' (Huenneke 1991), will be in greater danger of losing variation compared totaxa with originally restricted distributions (see also Barrett & Kohn 1991). The 'new rares' can be described as formerly wide-ranging taxa, with animal-mediated pollination and seed dispersal, which recently experienced a fragmentation of their distribution area by anthropogenic causes, and consequently decreased (or are still decreasing) in terms of numbers of populations and individuals. Many perennial herbs and forbs and shrubs belong to this category, particularly in mediterranean and temperate regions.

Fragmentation affects genetic variation

It may be questioned whether data on gene flow are relevant to the topic of conservation of wild relatives of European crops. Most of the data discussed were obtained from studies in natural, unstressed populations or in specially designed experimental settings. However, if we look at the current pattern in most parts of the world, long established patterns of distribution and continuity of spread are being broken up as a consequence of all kinds of land use and development. Europe may serve as an extreme example of this. Fragmentation of original landscapes is emanent, but itseffects on species and communities are far from being well understood. Some of the possible threats are mentioned by Opdam & Hengeveld (1990) and by Mader (1991). The components (subpopulations) of metapopulations are at risk of losing their genetic contacts. These subpopulations will generally suffer from a decrease in dispersal. Not only will gene flow between units diminish, but also the population size and density in each unit. This will result in higher extinction rates as a consequence of genetic drift and inbreeding depression (Elam & Ellstrand 1993), and also because of increased demographic and environmental stochasticity (Opdam & Hengeveld 1990, Menges 1991a). The chance of recolonization of a site will decrease. When the remnants of a habitat are small, environmental stochasticity will increase, and there may be accompanying changes in microclimate (Saunders & al. 1991), for example as a result of changes in the vegetation structure. Due to the decrease in the effective population size, genetic erosion may occur, and depending on the strategy of the species, the risk of inbreeding depression will grow (e.g. Templeton & al. 1990). The relationships between the apparent rate of gene flow (Govindaraju 1988), the distribution of genetic variation (Hamrick & al. 1991, 1995), and outcrossing rates (Barrett & Eckert 1990), found in comparing different life and pollination strategies, suggest that in particular the wide-ranging, perennial taxa are at risk (Hamrick & Godt 1989, Ellstrand & Elam 1993).

On the other hand, there are some suggestions that splitting up of a metapopulation into isolated, independent (eventually small) populations may on the long (evolutionary) run increase the genetic differentiation within a taxon (*Salvia pratensis* and *Scabiosa columbaria*: van Treuren & al. 1991, van Treuren 1993; *Senecio integrifolius*: Widén 1991, Widén & Andersson 1993), and furthermore could contribute to the survival by spreading the risk of extinction over a series of small sites (Templeton & al. 1990, Saunders & al. 1991).

However, we think that the consequences of the processes characteristic for small and isolated populations (inbreeding, loss of reproductive success, stochastic events, a.s.o.) will continue to bring high extinction risks.

Saunders & al. (1991) point out another set of possible consequences of fragmentation, i.e. the effects of the changing community on the individual species. Discontinuities will affect predator-prey relations, both with pathogens and herbivores. Plant-pollinator relationships will also be under stress. For example, Petanidou & al. (1995) found in *Gentiana pneumonanthe* that individuals growing in sites with a relatively high grass cover suffered from reduced reproductive success as a result of disturbed plant-pollinator interactions. In small habitat remnants, the species composition will change due to edge effects, invasions, or insufficient carrying capacity (Mader 1991).

Tscharntke (1992) describes the (local) extinction risks of a number of animal species in small remnant stands of *Phragmites australis*, the species to which they are essentially bound.

Incomplete knowledge

Saunders & al. (1991) stress in their review that tangible data on the effects of fragmentation are scarce, and propose points which need research:

(1) a comparison of pre- and postfragmented systems may give insight in the effects of external factors (such as changes in radiation and hydrology),

(2) changes in internal processes that have occurred since fragmentation should be studied,

(3) investigations of the rate of change (and loss) of genetic variation of isolated (and small) populations, and which effect a possible loss of genetic variation may have. Also the role of corridors for (restoring) movements in a fragmented landscape needs better understanding.

These authors emphasise that field studies and experiments will be particularly important because there is a direct need of management advice.

A considerable amount of current knowledge is gained from studies of (large) animal species. In plants, however, with their enormous diversity of breeding systems and life strategies, the effects of threat factors are far more difficult to assess (Templeton 1991). Menges (1991a) reviewed the special characters plants show with respect to the application of the minimum viable population concept. Some of these characters are: small neighbourhood size, microsite specialization, i.e. genecotypic differentiation, phenotypic plasticity (which may conceal genetic variation), clonal growth, spatial aggregations, and several aspects of the array of breeding systems.

In this respect, the effects of the following mechanisms (still) have a research priority.

(1) Population genetic effects from drift and founder events. From recent publications, the loss of fitness due to inbreeding depression increasingly appears to be important;

(2) Outbreeding depression and the possible loss of local adaptations in ecogeographical differentiation patterns (see below);

(3) Demographic stochasticity: in small, isolated populations, the small number of individuals may perform poorly by chance;

(4) Environmental stochasticity (unpredictability of the habitat, and different sorts of catastrophes as drought, flooding, frosts, see for example. Barrett & Kohn 1991).

(5) Reproductive biology *per se*: the reproductive success of specimens living in very small, and often isolated populations. It is also here that the fragmentation of the habitat plays an important role. The degree of isolation affects pollinator visitation rates, causing pollen limitation and thus reduced seed production (Jennersten 1988, Karron 1987, Ellstrand & Elam 1993, Lamont & al. 1993). Changes in the species' characteristic rate of outcrossing may follow (de Jong, Waser & Klinkhamer 1993, Klinkhamer & de Jong 1993, Raijmann & al. 1994), leading to increased levels of homozygosity and inbreeding depression (Oostermeijer & al. 1994).

Hybridization and outbreeding depression

A scarcely studied mechanism of extinction is hybridization between closely related species. In a review, Levin & al. (1996) show that particularly rare, insular species may be prone to extinction when hybridization with more common and ecologically generalistic relatives occurs. Apparently, this phenomenon occurs particularly in so-called ‘hybridized habitats’ and in fragmented landscapes with their inherent edge effects (Mader 1991).

We think that these conclusions also are true for subspecific taxa occurring in habitat islands. There, the negative effects of outbreeding depression may be responsible for the losses of biodiversity atthe level of ecotypic differentation. Between conspecific populations, outbreeding depression may occur when too remote (parts of) previously separated and differentiated ecotypes establish genetic contact. Particular genotypes can get lost by such genetic exchanges. Less fit recombinational genotypes may result from the disruption of co-adapted gene complexes. This phenomenon is known as outbreeding depression. Some authors found evidence for this mechanism in some species (Waser & Price 1989, 1991, Dudash 1990, Svensson 1990, Templeton 1991), while in other species it seems to be absent (Fenster & Sork 1988, Sobrevilla 1988, Oostermeijer & al. 1995a). How far this phenomenon occurs on a wider scale in plants should be investigated.In other words we know too little about outbreeding depression and what effect it might have. When attempts are made to improve genetic connections (connectivity) via corridors – ‘defragmentation’ — (as for example through ‘ecological infrastructure planning’, Anonymous 1990, 1991) or when species are reintroduced. As with the other mechanisms, this will not necessarily be restricted to rare species. The many examples of ecogeographical differentiation existing in wide-ranging species may also be prone to this sort of erosion. ‘Restoring’ gene flow where it was absent before may thus also decrease genetic diversity. Such management procedures should therefore be preceded by an analysis of the genetic consequences.

Fragmentation affects reproduction and fitness components

In this section, we will present some studies from the literature and from our own research, mainly related to reproductive biology. We will concentrate on species that were formerly widespread, but are now declining for some reason. Special attention will be given to cases of reduction in reproductive success and aspects of inbreeding depression. In case a taxon meets constraints in any part of its life strategy, the ultimate question is: does it do any harm, is its fitness negatively influenced?

Several studies concerning rare species occurring in small populations provide insight on the sort of problems they encounter. As explained before, the level of genetic variation may be low, but the question remains if this influences negatively reproductive success and consequently fitness. In most cases this is impossible to conclude from the general pattern. Wiens (1989), studying an endemic species of *Dedeckera*, showed that the seed/ovule ratio (seed set) was extremely low, suggesting that reproductive success is almost zero. However, this ultimately depends on the probability of the seeds’ becoming established.

Karron (1987, 1991), comparing narrowly distributed and wide-spread *Astragalus* species, found a strong pollen limitation in a rare taxon owing to the almost complete lack of pollinator visits. Various levels of pollen limitation may be quite common in nature, as a consequence of diverse factors (see for example, Campbell 1987, Johnston 1991).

Seed set in small populations

Recently, evidence is accumulating for the loss of reproductive capacity in small populations of (formerly) wide-ranging species due to pollen limitation, be it by the degree of isolation, or by the absence of the (specialized) pollinators from the habitat. Jennersten (1988) reported on loss of seed set in *Dianthus deltoides*. Based on a study of *Melampyrum arvense*, Kwak (1988) stresses the importance of facilitating stepping stones (in the form of patches of other flowering species) for populations that have become isolated. Kwak & al. (1991) record a total absence of seed set in *Phyteuma nigrum* in small, isolated populations. No such facilitating stepping stones were present in a circle of 250 metres around the site.

We studied, among others, several Dutch *Gentiana* species, and found loss of seed set in small populations of *G. pneumonanthe* (Oostermeijer & al. 1992, Oostermeijer 1996) and of *G. cruciata* (Petanidou & al. 1991, 1995). In *Gentianella ciliata*, only approximately 2 % seed set was found in the only, and therefore highly isolated, population growing in The Netherlands. The biennial *G. germanica* was very poorly pollinated in a small, isolated site and seed set at this site seems to be the result of spontaneous selfing (Luijten, pers. comm.). A study on *Arnica montana* in The Netherlands, where this species is increasingly rare, showed self-incompatibility and clonal growth, resulting in a genetically clustered structure of the population (Luijten & al. 1996). A strong decrease was observed in both seed set and seedling performance as population size declines (Luijten, den Nijs & Oostermeijer, unpublished results). Besides possibly reduced visitation rates, this strong effect may be attributed to the loss of self-incompatibility alleles in small populations (Byers & Meagher 1992).

All these species that were formerly widespread and mostly formerly rather common, now occur in fragmented population complexes, undergoing increasing isolation and decreasing population size. The consequence of these three factors is reduced seed set and lower seed quality (at least partly as a result of inbreeding), affecting fitness negatively. Oostermeijer (1996) showed that in small populations of *Gentiana pneumonanthe* it was not the reduced seed set *per se*, but the additional effect of inbreeding depression that increased the extinction likelihood substantially. These data indicate the important role pollination ecology may have in maintaining the viability of a population. Corbett & al. 1991, in a survey on bees and the pollination of crops and wild flowers in the European community, conclude that while there is much known on the management of the pollination of crops, but that the importance of insect pollination and its adequacy in wild species is poorly studied, they state that it is an essential factor for survival. Osborne & al. (1991) therefore supposed that limited pollination may ultimately lead to extinction. These authors, and in a connected study also Williams & al. (1991) emphasize the need of a coordinated research programme to integrate pollination into conservation strategies.

Inbreeding effects

Menges (1991b) reports reduced germination in seeds obtained from small populations of *Silene regia*. He suggested two alternative explanations: pollen limitation and inbreeding depression. In self-compatible species, one of the direct consequences of reduced pollinator visits is the increase of the amount of selfing and near-neighbour mating, leading to inbreeding, and inbreeding depression. To what extent this really affects wild plant populations is still scarcely studied, – only recently are more data becoming available.

Measuring progeny survival and reproduction (fruit mass and number), Dudash (1990) found a very significant inbreeding depression in the first generation after selfing *Sabatia angularis (Gentianaceae)* individual plants in a large, 'abundant' population. Reduced fitness (decrease in seed set, germination, juvenile survival, and reproductive output) was also found in *Chamaecrista (Leguminosae),* maximally after complete selfing, and to a lesser degree with increasing distance of the pollen donor (Fenster 1991). Barrett & Kohn (1991) found significant effects in the very widespread, outcrossing *Eichornia paniculata.* A strong decrease in relative fitness of inbred progenies occurred also in *Mimulus* species (Dole & Ritland 1993). Ouborg (1993) found a 40-75 % loss in relative performance of selfed offspring of *Salvia pratensis* and *Scabiosa columbaria.* In *Lychnis flos-cuculi,* Hauser & Loeschke (1994) found higher abortion rates, lighter seeds, lower germination speed, and a lower germination percentage in the second generation of selfing.

In addition, our studies on *Gentiana pneumonanthe* revealed inbreeding depression in small, declining natural populations in The Netherlands. Here there is a significant increase in the selfing rate with decrease of population size, a positive correlation between the proportion of polymorphic loci and population size, and hardly any gene flow between populations (Raijmann & al. 1994). Measuring fitness components in this series of populations provided insight into the loss of fitness in small populations. Mean seedling weight, total progeny yield, flowering performance and adult weight were all positively correlated with population size (Oostermeijer & al. 1994, Oostermeijer 1996).

Analysis of the genotypic constitution of these possibly inbred progenies revealed to a strong correlation between the level of heterozygosity and plant performance. A positive correlation was found between the number of heterozygous loci in a given individual and several fitness parameters, such as seedling weight, adult weight, and the number of flowers produced per plant (Oostermeijer & al. 1995b). Similarly, heterozygote advantage for several fitness parameters in plants has been demonstrated by Schaal & Levin (1976), Linhart & Mitton (1985), Wolff & Haeck (1990), and Tomekpe & Lumaret (1991).

From data gathered such as these, it is evident that fragmentation has a negative impact on reproductive success. Consequently, the accompanying loss of genetic variation and inbreeding depression in small populations will contribute to the decrease in the population's chances of survival.

Conclusions

Widespread and common species (particularly when cross-pollinated) suffer more from fragmentation and isolation than endemic and naturally rare species. Conservation efforts, should therefore be directed more to the category of species that recently became rare. This is the more so since at preswent priority for the conservation of newly rare species is mostly low, because they are frequenetly regarded as still widely distributed and 'relatively common'.

Monitoring of reproductive success, performance, and recruitment from large as well as small and isolated populations of selected (wild) species, particularly self-compatible and normally outbreeding herbs will help develop a conservation strategy. These enquiries should be accompanied by analyses of the genetic constitution within and among populations. As pollination is an essential factor for successful reproduction, it tooshould receive more attention. In particular the suggested inferior performance of honey bees in the pollination of wild flowers (Wilson & Thomson 1991, Stanton & al. 1991, Corbett & al. 1991, Westerkamp 1991, Stanton & al. 1992) deserves attention. The increased use of managed honey bees over the past years, for the pollination of crops, or just for honey production, may interfere with natural insect pollination. This may lead to losses in reproductive success, due to the relative inefficiency of honeybee pollination and the changes in competitive relations within the insect species community (Williams & al. 1991).

The preparation of an ecological infrastructural network (EECONET) and ecological restoration programmes in general should take all these aspects into consideration.

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