F. X. Picó, C. Mix, N. J. Ouborg, & J. M. van Groenendael

# Might family-level variation in inbreeding depression have implications for the conservation of fragmented plant populations?

**Abstract**

Picó, F. X., Mix, C. N., Ouborg,, J. & van Groenendael, J. M. : Might family-level variation in inbreeding depression have implications for the conservation of fragmented plant populations? — Bocconea 16(1): 173-181. 2003. — ISSN 1120-4060.

Current distributions of many European plants are reduced to a mosaic of small isolated populations which have to face the demographic and genetic consequences of habitat fragmentation. Apart from environmental and demographic stochasticity, inbreeding depression is considered as an important factor determining extinction risks of fragmented plant populations. Despite the important theoretical background regarding the evolutionary dynamics of inbreeding, there is a considerable lack of empirical data to understand how real plant populations respond to inbreeding. In this paper, we suggest that individual-level variation in inbreeding depression may play a significant role in decreasing extinction probabilities of fragmented plant populations, as not all individuals within a population can be negatively affected by inbreeding depression. We present an overview about conservation genetics, paying special attention to the theoretical significance of family-level variation in inbreeding depression for plant conservation biology. We illustrate theory with preliminary results of two ongoing research projects focusing on the conservation of plant species in fragmented habitats.

## Introduction

Many plants that were commonly distributed in Europe some decades ago are considered seriously at risk today or have gone extinct in recent years, as reported for the Netherlands (Weeda & al. 1990), Germany (Korneck & al. 1996), or Switzerland (Landolt 1991). As a result of severe habitat fragmentation, the occurrence of many plant species is reduced to small isolated populations, a fact with important demographic and genetic consequences (Young & Clarke 2000). Extinction probabilities of small isolated populations of plants can be thought to be determined at three hierarchical levels (Tilman & al. 1994; Schemske & al. 1994): (1) large-scale dynamics of populations defined by colonisation and extinction rates (metapopulation dynamics), (2) persistence and dispersal capacity of local populations defined by birth/death and dispersal rates (population dynamics), and (3) variation among individuals in traits concerning persistence and dispersal capacity as a result of selective pressures (trait dynamics).

Changes in persistence and dispersal traits at the individual level deserves special attention, as their ultimate effects can determine the dynamics of the metapopulation as a whole. Fragmentation reduces the number of breeding individuals within a population while reducing gene flow between populations. As a result, inbreeding increases resulting in inbred progeny that may suffer from inbreeding depression, the relative loss of fitness due to inbreeding. Continued inbreeding within a population may lead to decreased genetic diversity which can be related to increased extinction probabilities (Booy & al. 2000; Dudash & Fenster 2000). In this paper, we present an overview about the implications of inbreeding depression for the conservation of plant populations. In particular, we summarise the theory pertaining to inbreeding depression, and stress the importance that among-individual variation in inbreeding depression may have to understand how populations respond to inbreeding. Based on two ongoing research programmes, FLOS and TRANSPLANT which aim at quantifying extinction risks of fragmented plant populations, we finally present preliminary results on the extent of between- and within-population variation in inbreeding depression.

## The genetic basis of inbreeding depression

There are two main hypothesis for the genetic basis of inbreeding depression which predict similar declines in inbred fitness (see Charlesworth & Charlesworth 1987). Under the 'overdominance hypothesis', outbred progeny perform better than inbred progeny because the former have a larger proportion of heterozygous loci which mostly are overdominant. According to the 'partial dominance hypothesis', which is supported by some genetic analyses (Johnston & Schoen 1995; Dudash & Carr 1998; but see Fu & Ritland 1994), outbred progeny also perform better than inbred progeny but in this case because inbred individuals are more homozygous for deleterious recessive alleles. Under this last model, inbreeding depression is expected to be higher in outbred populations, because deleterious recessive mutations are accumulated, while inbreeding depression should be lower in inbred populations, as deleterious recessive mutations are exposed and selected against (see examples in Holtsford & Ellstrand 1990; Dole & Ritland 1993; Carr & Dudash 1996; Johnston & Schoen 1996; Affre & Thompson 1997).

Under this scenario, two evolutionary stable endpoints combining mating system and inbreeding depression are expected. On the one hand, predominant outcrossing with strong inbreeding depression, and on the other hand, predominant self-fertilization associated with weak inbreeding depression, because of purging of highly deleterious alleles (Lande & Schemske 1985; Charlesworth & al. 1990). However, plant natural systems appear to be more complex given the wide existing diversity of mating systems. Theoretical studies have emphasised the role that within-population variation (i.e. family-level variation) in inbreeding depression can play in explaining the existence of partially self-fertilizing species or whether a selfing variant can invade in an outcrossing population (Holsinger 1988; Uyenoyama & Waller 1991a,b,c; Schultz & Willis 1995). These models indicate that associations between viability and mating-system loci may be more relevant in predicting the evolution of mating systems within a population than population-level inbreeding depression and mating-system estimates. Recent detailed studies have addressed this issue in depth providing empirical support for the existence and importance of variation in inbreeding depression among maternal plants to understand mating-system evolution (Koelewijn 1998; Dudash & al. 1997; Mutikainen & Delph 1998).

## Inbreeding depression and the survival of populations

The direct consequence of habitat fragmentation is the reduction of population size and increased isolation among populations. The extinction probabilities of small isolated plant populations tend to increase because of several environmental, demographic, and genetic factors that may act in concert. For example, the attractiveness to pollinators of small isolated populations can be enourmously reduced (Groom 2001), decreasing gene flow and increasing genetic drift and inbreeding which decrease plant fitness through inbreeding depression (Ellstrand & Elam 1993). The susceptibility of small populations to environmental and demographic stochasticity tends to increase, altogether determining population extinction probabilities (Menges 1990). Several factors, such as mating system, population size, degree of population isolation, the extent of gene flow, and population history can affect the extent of inbreeding depression (see Ellstrand & Ellam 1993), and consequently the extinction probability estimates of plant populations.

From a conservational point of view, family-level (i.e. among individuals within a population) variation in inbreeding depression might play a significant role in the persistence of plant populations in fragmented landscapes. Significant variation among individuals in inbreeding depression, in such a way that some inbred families outperformed their outcrossed relatives, implies that selfing variants, and their progeny produced by recurrent self-fertilization, could be important for population maintenance. That is, after dramatic episodes of habitat fragmentation, large plant populations becoming small inbred populations would experience important changes in genetic composition and mating system of extant plants but without risk of going extinct. Large variation among families in inbreeding depression could enhance the persistence in fragmented habitats, specially for plants with partially self-fertilizing mating systems occurring in fragmented landscapes where gene flow between populations can be scarce. Significant among-individual variation in inbreeding depression after one or more generations has been found in some studies (Schemske 1983; Sakai & al. 1989; Agren & Schemske 1993; Ouborg & van Treuren 1994; Husband & Schemske 1995; Ouborg & al. 2000), but the importance of this result for the long-term persistence of populations has not yet been assessed. On a metapopulation basis, the extent of family-level variation in inbreeding depression might be even more relevant. So far, the entire metapopulation dynamics can be highly determined by which genotype (characterised by the positive or negative response to inbreeding) colonises suitable vacant sites, since after colonization inbreeding will be a prominent feature.

## Flos and Transplant programmes

Two ongoing research projects are evaluating the effect of habitat fragmentation on the survival of plant populations. FLOS focuses on this topic at a regional scale while TRANSPLANT addresses the same questions over a wider geographical scale. Study plant species have been chosen in such a way that contrasted longevity (i.e. short- and long-lived plants) and dispersability (i.e. low and high capacity), traits with particular relevance for population persistence and colonization of suitable sites, respectively, are represented (Table 1). Hence, these projects will allow to determine how different population sizes and degrees of isolation, which in turn lead to increased inbreeding, affect the persistence of plant species with different life histories and dispersal capacities. Preliminary results concerning the effects of inbreeding for *Succisa pratensis* Moench, *Hypochaeris radicata* L. and *Tragopogon pratensis* L. are presented. In particular, we emphasise on the significant among-individual variation found in inbreeding depression for seed mass and seed germination rate. Inbreeding depression is tested among families comparing mean seed mass and mean seed germination rate between pollination treatments: (1) selfing (self-pollination), (2) outcrossing within populations (cross-pollination with pollen of plants from the same population), and (3) outcrossing between populations (cross-pollination with pollen of plants from another population).

Representative results from Dutch populations (from a total of eight populations) of *S. pratensis* and *H. radicata* are illustrated in Fig. 1. For both seed mass and seed germination rate, two groups of families can be observed: individuals that yielded inbred progeny that outperformed outbred progeny, and vice-versa. The same type of families were found in all study populations. Interestingly, the distance from the pollen donor (i.e. from plants within or among populations) also affected the performance of some families for both plant species, indicating the susceptibility of families to the pollen source. The effects of inbreeding on the same dependent variables of study for two German populations of *T. pratensis* are shown in Fig. 2. In this case, family-level variation in inbreeding depression (comparing only selfing and outcrossing within population treatments) is more apparent for seed germination rate than for seed mass. Overall, these results indicate that differential responses to inbreeding of individual plants within a population become readily perceivable in early stages of the life cycle. If fragmentation increases inbreeding, populations of these three plant species seem to have the genetic potential to cope with the effects of inbreeding depression. It is obvious that population-level estimates of inbreeding depression are meaningless when among-individual variation in inbreeding depression is so sig-

Table 1. Study plant species selected in the FLOS(1) and TRANSPLANT(2) programmes to study the effects of inbreeding depression on progeny performance. Species fulfil the longevity-dispersability matrix.

| | Longevity | |
|---|---|---|
| Dispersability | Short-lived | Long-lived |
| Low | *Scabiosa columbaria* L. (2) | *Succisa pratensis* Moench (1,2) |
| High | *Tragopogon pratensis* L. (2) | *Hypochaeris radicata* L. (1,2) |

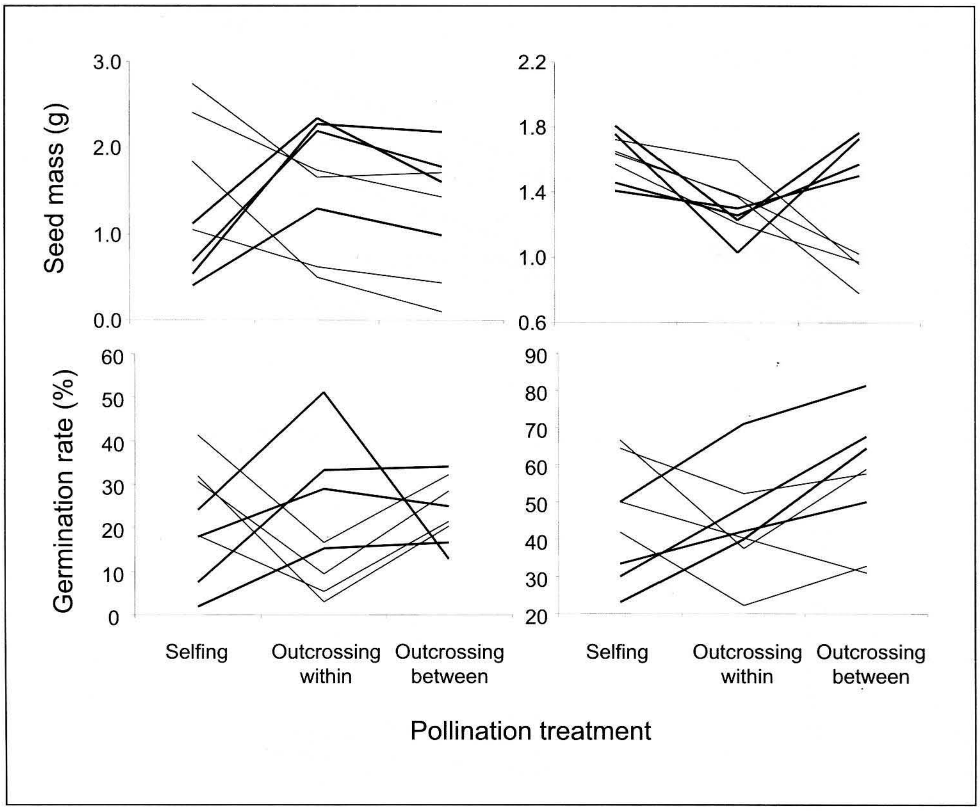

Fig. 1. Mean seed mass (upper panels) and mean germination rates (lower panels) for representative families within populations in different pollination treatments for *Succisa pratensis* (left panels) and *Hypochaeris radicata* (right panels). Thicker and thinner lines correspond to individuals with similar responses to inbreeding.

nificant, as the potential of plant populations to undergo the effects of fragmentation is underestimated.

## Ongoing research

Further studies are needed to assess the real extent of family-level variation in inbreeding depression. According to Byers & Waller (1999), family-level variation in inbreeding depression, indicated by significant maternal family by cross interactions, reflect the potential for greater selfing rates to coevolve with purging within populations. However, the genetic basis of such empirical evidence requires further investigation. Although simulation models illustrate the risk of population extinction from fixation of deleterious mutations in small populations (Lande 1994), other theoretical studies (see Holsinger 1988 and references therein) show that increased selfing rates can be favoured if the fitness of inbred progeny and the genotype at loci determining mating-system are associated. This is

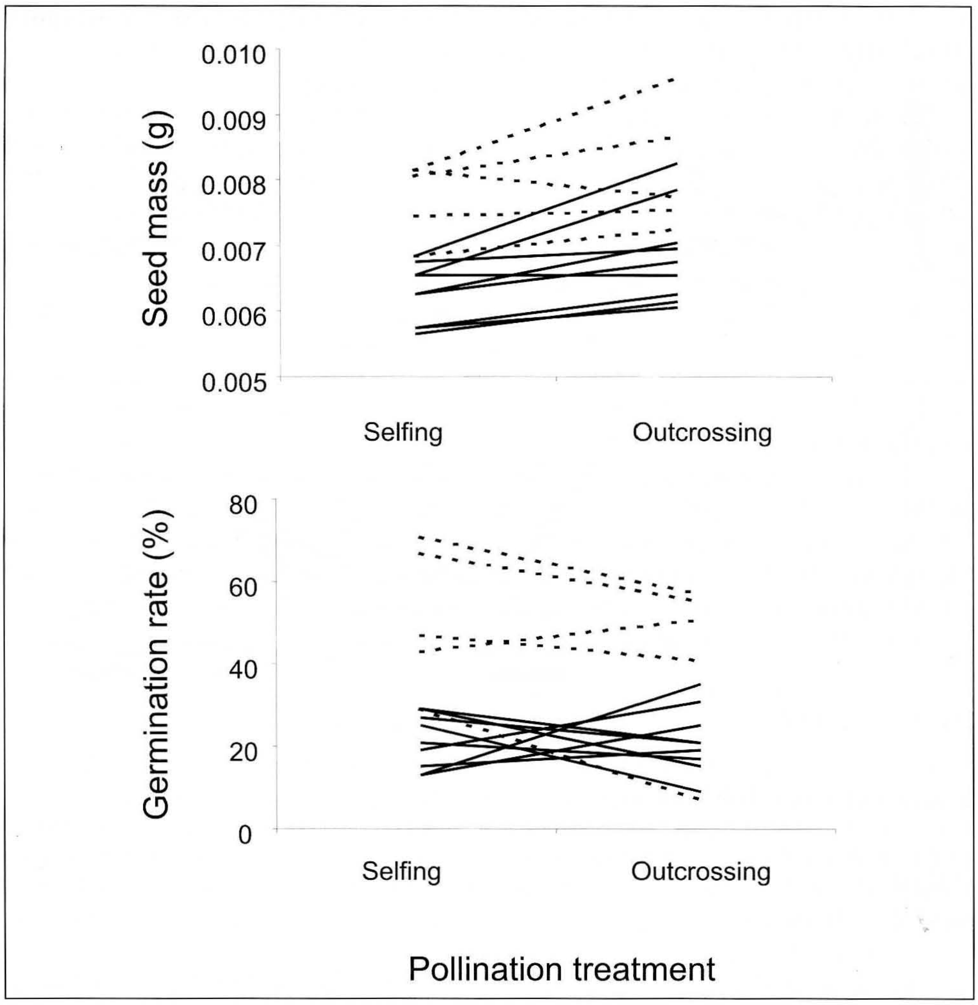

Fig. 2. Mean seed mass and mean germination rates for different families in different pollination treatments for *Tragopogon pratensis*. Solid and dotted lines represent individuals within the same population.

an interesting theoretical result, as individuals homozygous or heterozygous at mating-system loci are likely to be also homozygous or heterozygous at fitness locus, which would explain why inbred and outbred progeny seem to benefit from selfing and outcrossing, respectively.

Strong evidence for the significance that family-level variation in inbreeding depression can have for the survival of plant populations, must be obtained by comparing absolute fitnesses (i.e. from seed germination to reproduction per maternal line) between inbred and outbred progeny. The reason is that strongly deleterious alleles can be easily purged from the population in early phases of development, whereas weakly deteleterious alleles can result more difficult to purge and can be expressed in later stages (Husband & Schemske 1996). Hence, the extent of variation among families in inbreeding depression must be evaluated by taking into account the entire individual's life cycle.

Finally, it will be very interesting to get more insights into the relationship between the inbreeding level and inbreeding depression at an individual level (but see Koelewijn 1998; Mutikainen & Delph 1998). If fitness and mating-system loci are associated, then both positive and negative relationships between the level of inbreeding and inbreeding depression are expected in function of the response of individual plants to inbreeding depression, indicating that plants within a population would be capable of evolving to either direction, i.e. high levels of inbreeding with weak (selfing plants) and with strong (outcrossing plants) inbreeding depression. Finding out such relationships is not a straightforward task given the fact that inbreeding depression seems to be a non-linear function of inbreeding level as a result of the genetic complexity shown by many traits (Ouborg & al. 2000 and references therein).

All this knowledge will be very useful to address problems concerning the conservation of fragmented plant populations. In particular, demographic models (based on empirical data) accounting for the effects of environmental and demographic stochasticity could also include the individual-level variation in inbreeding depression (see Oostermeijer 1996 for a population-level approach), simulating therefore the genetic effects of decreased population size and increased isolation.

## Acknowledgements

This research reported here is supported by NWO (Netherlands Organisation for Scientific Research; Flos: 805-33-451) and the European Union (Transplant: EVK2-1999-00004).

## References

Affre, L. & Thompson, J. D. 1997: Variation in the population genetic structure of two *Cyclamen* species on the island of Corsica. — Heredity **78**: 205-214.

Ågren, J. & Schemske, D. W. 1993: Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. — Evolution **47**:125-135.

Booy, G., Hendriks, R. J. J., Smulders, M. J. M., van Groenendael, J. M. & Vosman, B. 2000: Genetic diversity and the survival of populations. — Plant Biol. **2**: 379-395.

Byers, D. L. & Waller, D. M. 1999: Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. — Annu. Rev. Ecol. Syst. **30**: 479-513.

Carr, D. E. & Dudash, M. R. 1996: Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female functions. — Heredity **75**: 437-445.

Charlesworth, D. & Charlesworth, B. 1987: Inbreeding depression and its evolutionary consequences. — Annu. Rev. Ecol. Syst. **18**: 237-268.

— , Morgan, M. T. & Charlesworth, B. 1990: Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. — Evolution **44**: 1469-1489.

Dole, J. & Ritland, K. 1993: Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. — Evolution **47**: 361-373.

Dudash, M. R. & Carr, D. E. 1998: Genetics underlying inbreeding depression in *Mumulus* with contrasting mating systems. — Nature **393**: 682-684.

— , Carr, D. E. & Fenster, C. B. 1997: Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. — Evolution **51**: 54-65.

— & Fenster, C. B. 2000: Inbreeding and outbreeding depression in fragmented populations. — Pp. 35-54 in: Young, A. G. & Clarke, G. M. (eds.), Genetics, demography and viability of fragmented populations. — Cambridge.

Ellstrand, N. C. & Ellam, D. R. 1993: Population genetic consequences of small population size: Implications for plant conservation. — Annu. Rev. Ecol. Syst. **24**: 217-242.

Fu, Y. B. & Ritland, K. 1994: Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. — Genetics **136**: 323-331.

Groom, M. J. 2001: Consequences of subpopulation isolation for pollination, herbivory, and population growth in *Clarkia concinna concinna* (*Onagraceae*). — Biol. Conserv. **100**: 55-63.

Holsinger, K. E. 1988: Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. — Evolution **42**: 1235-1244.

Holtsford, T. P. & Ellstrand, N. C. 1990: Inbreeding effects in *Clarkia temblorensis* (*Onagraceae*) populations with different natural outcrossing rates. — Evolution **44**: 2031-2046.

Husband, B. C. & Schemske, D. W. 1995: Magnitude and timing of inbreeding depression in a diploid population of *Epilobium augustifolium* (*Onagraceae*). — Heredity **75**:206-215.

Johnston, M. O. & Schoen, D. J. 1995: Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. — Science **267**: 226-229.

— 1996: Correlated evolution of self-fertilization and inbreeding depression: an experimental study of nine populations of *Amsinckia* (*Boraginaceae*). — Evolution **50**: 1478-1491.

Koelewijn, H. P. 1998: Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. — Evolution **52**: 692-702.

Korneck, D., Schnittler, M. & Vollmer, I. 1996: Rote liste der Farn- und Bluetenpflanzen (*Pteridophyta* et *Spermatophyta*) Deutschlands. — Schriftenreihe fuer Vegetationskunde **28**: 21-187.

Lande, R. 1994: Risk of population extinction from fixation of new deleterious mutations. — Evolution **48**: 1460-1469.

— & Schemske, D. W. 1985: The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. — Evolution **39**: 24-40.

Landolt, E. 1991: Gefaerdung der Farn- und Bluetenpflanzen in der Schweiz. Bundesamt fuer Umwelt, Wald und Landschaft. — Bern.

Menges, E. S. 1990: Population viability analysis of an endangered plant. — Conserv. Biol. **4**: 41-62.

Mutikainen, P. & Delph, L. F. 1998: Inbreeding depression in gynodioecious *Lobelia siphilitica*: Among-family differences override between-morph differences. — Evolution **52**: 1572-1582.

Oostermeijer, J. G. B. 1996: Population viability of the rare *Gentiana pneumonanthe*. The relative importance of demography, genetics, and reproductive biology. Dissertation. — Amsterdam.

Ouborg, N. J. & van Treuren, R. 1994: The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. — Evolution **48**:996-1008.

— , Biere, A. & Mudde, C. L. 2000: Inbreeding effects on resistance and transmission-related traits in the *Silene–Microbotryum* pathosystem. — Ecology **81**: 520-531.

Sakai, A. K., Karoly, K. & Weller, S. G. 1989: Inbreeding depression in *Schiedea globosa* and *S. salicaria* (*Caryophyllaceae*), subdioecious and gynodioecious Hawaiian species. — Am. J. Bot. **76**: 437-444.

Schemske, D. W. 1983: Breeding system and habitat effects on fitness components in three neotropical *Costus* (*Zingiberaceae*). — Evolution **37**: 523-539.

— , Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M. & Bishop, J. G. 1994: Evaluating approaches to the conservation of rare and endangered plant species. — Ecology **75**: 584-606.

Schultz, S. T. & Willis, J. H. 1995: Individual variation in inbreeding depression: the roles of inbreeding history and mutation. — Genetics **141**: 1209-1223.

Tilman, D., May, R. M., Lehman, C. L. & Nowak, M. A. 1994: Habitat destruction and the extinction debt. — Nature **371**: 65-66.

Uyenoyama, M. K. & Waller, D. M. 1991a: Coevolution of self-fertilization and inbreeding depression. I. Mutation-selection balance at one and two loci. — Theor. Popul. Biol. **40:** 14-46.

— 1991b: Coevolution of self-fertilization and inbreeding depression. II. Symetric overdominance in viability. — Theor. Popul. Biol. **40**: 47-77.

— 1991c: Coevolution of self-fertilization and inbreeding depression. III. Homozygous lethal mutations at multiple loci. — Theor. Popul. Biol. **40**: 173-210.

Weeda, E. J., van der Werf, A. & Bakker, P. 1990: Rode lijst van de in Nederland verdwenen en bedreigde planten (*Pteridophyta* en *Spermatophyta*) over de periode 1.1.1980-1.1.1990. — Gorteria **16**.

Young, A. G. & Clarke, G. M. 2000: Genetics, demography and viability of fragmented populations. — Cambridge.

Addresses of the authors:
F. X. Picó, C. Mix, N. J. Ouborg, & J. M. van Groenendael, Department of Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmgen, the Netherlands.
Corresponding author: Phone: +31 24 3652521.
E-mail: xavier.pico@sci.kun.nl