Genetic introgression from distant provenances reduces fitness in local weed populations

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Summary

1. Seed mixtures of wildflowers are used increasingly in schemes to restore biodiversity in intensively managed farmland. Usually, the seed mixtures are produced by commercial suppliers and they may be distributed over large geographical distances. It is therefore important to ask what problems may arise from using seed that is not of local origin. The aim of this study was to evaluate one potential problem, namely the effects of genetic introgression of foreign provenances on the fitness of local weed populations.

2. The problem was investigated using the arable weed species *Agrostemma githago*, *Papaver rhoeas* and *Silene alba*, all of which are commonly included in commercial seed mixtures in Switzerland. Hybrids (F_1 and F_2 backcrosses) were made between local Swiss plants and plants of English, German and Hungarian provenance (plus F_1 from one US source in *Silene*). In a field experiment the growth of the hybrid plants was compared with that of the parents. Above-ground dry matter after one growing season was taken as a measure of fitness. Additionally, survivorship and seed mass were determined for some of the hybrids.

3. The biomass data revealed negative outbreeding effects caused by epistasis in all four F_2 backcrosses of *Papaver* and in the F_2 of *Agrostemma* hybridized with plants of German provenance; no such effects were found in *Silene*. Survival was slightly lower in the F_1 hybrids of *Papaver*, and considerably reduced in the F_2 backcrosses. For *Silene*, a heterosis effect was evident in seed mass in the F_1 generation, while seed mass decreased in the F_2. The same trend, although less strong, was also observed in *Agrostemma*.

4. The results suggest that only plants of relatively local origin should be used in wildflower mixtures, although it is not possible to specify precisely over what distance seed can safely be transferred. The same recommendation is also valid for schemes to reinvigorate endangered plant populations. The relevance of the fitness components that were measured, and the long-term effects of genetic introgressions, are discussed.

Key-words: epistasis, heterosis, mortality, outbreeding depression, plant biomass.

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Introduction

WILDFLOWER STRIPS

The reduction in the proportion of non-cultivated land and fallow fields in the rural landscapes of western and central Europe has led to substantial losses in habitat diversity and to a decline of many species that are characteristic of traditional agricultural landscapes (Aebischer 1991; Potts 1991; Andreasen, Stryhn & Streibig 1996; Siriwardena *et al.* 1998, 2000; Hald 1999). The undesirable consequences of these trends were recognized in the 1980s, and increasing concern for environmental quality has led to a shift in agricultural policy away from the dominating priority to maximize yields. One product of this shift has been a variety of schemes to promote the restoration of species-rich habitats such as hedgerows and field margins (Aebischer, Blyke & Boatman 1994; Corbet 1995; Feber, Smith & Mac-

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donald 1996). In Switzerland, planted wildflower strips have become important habitat elements within the agricultural landscapes and have been shown to benefit the adjacent crops (Wyss 1995) and to enhance biodiversity (Frei & Manhart 1992; Greiler 1993; Frank 1996). For such wildflower strips, special seed mixtures are necessary because in intensive agro-ecosystems the soil seed bank is often depleted (Albrecht & Forster 1996).

As in other restoration projects, questions arise about the quality of plant material used to create such wildflower strips, and the distances over which plant genetic material should be introduced (Fenster & Dudash 1994; Hodder & Bullock 1997; Knapp & Rice 1998; van Andel 1998; Manchester & Bullock, 2000). Concerns about such planting and landscaping programmes have been discussed in the UK (Akeroyd 1994) and in the USA (Millar & Libby 1991; Reinartz 1995; Guerrant 1996; Montalvo *et al.* 1997). Besides maladaptation of foreign plants, which causes decreased germination rates and poor establishment, or dysfunction of plant–animal interactions (Keller & Kollmann 1998, 1999; Keller, Kollmann & Edwards 1999), genetic introgression of foreign genes is also a potential threat to native populations. Even though the sown vegetation is commonly removed after 2–6 years, and permanent establishment of introduced plants in the field or at the field margin has rarely been observed (Ramseier 1994), the increasing abundance of wildflower strips increases the risk of genetic introgression. The threat of introducing alien genes into local populations lies not only in their poor adaptation to local conditions, but also in their possible destabilizing effects on the genetic integrity of native populations. It could be argued that, in the absence of further genetic disruption, natural selection will lead to a recovery in fitness over time (Emlen 1991). However, this argument may not apply in the case of the plant species that are sown in wildflower strips, because they are repeatedly introduced over several years and thus continue to challenge the native populations.

OUTBREEDING DEPRESSION

Outbreeding depression describes the manifestation of reduced fitness in hybrid offspring. There appear to be two main causes for such effects, namely (i) the loss of local adaptation and (ii) the loss of intrinsic co-adaptation (Price & Waser 1979; Templeton 1986). The first one, which can be called the environmental component, is caused by inherited traits that are not well adapted to the new environment (Roff 1998). This effect is likely to increase with increasing environmental differences between the parental origins and usually cannot be detected under controlled conditions in a greenhouse or a growth chamber, as argued by Waser (1993) and Waser & Price (1994).

The other mechanism for outbreeding depression, the physiological component (Roff 1998), is caused by the disruption of co-adapted gene complexes. This component of outbreeding depression, also referred to as epistasis or hybrid breakdown, is detectable under controlled conditions but is not necessarily evident before the F_2 (Wallace 1955). Indeed, Lynch (1991) provided a hypothetical model that includes the various effects of gene actions, showing that 'F_1 progeny of distant parents always have enhanced fitness relative to progeny of contiguous parents'. The cause of epistasis in the F_2 became evident with the observation of Brncic (1954) that the 'breakdown is a result of intrachromosomal recombination between chromosomes of diverse origins'. While some decades ago epistasis was considered to occur only in species with restricted chromosomal recombination (Templeton 1986), there is now evidence that genetic interactions and the resulting outbreeding depression are a common phenomenon (Whitlock *et al.* 1995). For example, Hauser, Shaw & Østergård (1998) reported that first generation (F_1) hybrids of *Brassica napus* and *B. rapa* are intermediate in fitness between the two parents, whereas second generation (F_2) hybrids and backcrosses ($F_1 \times$ either parent) have a reduced fitness relative to both parents (Hauser, Jørgensen & Østergård 1998).

Although it is difficult to distinguish the two components of outbreeding depression without undertaking reciprocal transplant studies (van Andel 1998), the performance of the alien parent plants under the new environmental conditions provides some indication of the degree of maladaptation. Maladaptation may also be evident in the F_1, although in this generation it is often partly or wholly compensated for by heterosis. In subsequent generations the hybrids are likely to be increasingly purged of bad genes through selection, especially when there is backcrossing within the local population. Epistasis, on the other hand, may increase in subsequent generations, because interacting loci are separated with every mating (Wallace 1955). This tendency has been demonstrated experimentally and also predicted from simulations (Campbell & Waser 1987).

For any particular species there is likely to be a critical spatial separation between individuals at which outcrossing begins to reduce fitness. Between systematically distinct units (e.g. subspecies) epistatic effects may even lead to inviability or infertility in the offspring generations (Barton & Hewitt 1982; Templeton 1986; Coyne & Orr 1989), especially if the chromosomal sets of mates differ in structure and number (Barton & Hewitt 1982; de Boer 1982). For conspecific populations, the effect of genetic introgression may vary considerably depending on life-history traits, mating system and other traits that influence gene flow (Hamrick, Lin-

hart & Mitton 1979; Montalvo *et al.* 1997). It has been suggested that species with a high tendency for selfing, and small or fragmented populations with low dispersal of pollen and seeds, are more sensitive to outbreeding depression than widespread, well-dispersed species and outbreeders (Waser & Price 1983; Fenster & Dudash 1994).

DESIGN AND OBJECTIVES OF THE STUDY

To evaluate whether outbreeding depression occurs in those widespread agricultural weeds that are planted in wildflower strips, hybrid progenies of crossings with parents from other European countries were investigated in a field experiment. In such experiments, it is important to test the performance of genotypes under natural conditions, as fitness is a function of genotype, environment and the genotype–environment interaction (Griffing & Zsiros 1971; Mitchell-Olds 1986; Shaw 1986).

The crossing experiment was designed to simulate a single introgression by foreign pollen, as gene flow by pollen often exceeds the effect of seed dispersal (Ellstrand 1992). For this reason, the crossings were in one direction, the local Swiss provenance being the mother plants; thus the introgressions only interfered with the nuclear genome. This asymmetrical design was chosen deliberately as the study had the applied objective of investigating how genetic introgression affects native populations. The fundamental questions of genetic architecture and the mechanisms for outbreeding depression were not addressed. Evidence for genetic breakdown is assumed when the F_2 shows a fitness of less than the average between parents and the F_1 (Fenster & Dudash 1994; Whitlock *et al.* 1995).

The following specific questions were addressed in this study.
1. Do hybrids of agricultural and ruderal weeds with distant provenances show increased performance in the F_1 generation, i.e. heterosis?
2. Do the same hybrids show decreased fitness in the F_2, i.e. outbreeding depression?
3. What are the relative magnitudes of the ecological and physiological components of outbreeding depression?
4. To what extent are heterosis and outbreeding depression related to the geographical distance of the foreign population?
5. Is the rare inbreeding species *Agrostemma githago* more affected than the two ruderal outbreeders *Papaver rhoeas* and *Silene alba*?

Materials and methods

PLANT MATERIAL

Three agricultural weeds, *Agrostemma githago*, *Papaver rhoeas* and *Silene alba* (= *S. latifolia* ssp. *alba*), were used for the crossing experiment. Information on life history and mating system is given in Table 1. The plant names follow Tutin *et al.* (1964–80); only generic names are used subsequently.

Seed was obtained from commercial suppliers in Switzerland (FENACO, Winterthur), Germany (C. Appel GmbH, Darmstadt), England (Emorsgate seeds, King's Lynn, Norfolk) and Hungary (Szilas Plant KFT, Kereps). In most cases the suppliers provided information on the seed provenance to the level of county or district (Keller, Kollmann & Edwards 1999). In order to ensure the genetic quality of seed, some companies collect seeds from wild populations or at least replace the seed stock every 3–4 years with new wild material. The Swiss seed was the same as that which is sown in wildflower strips in Switzerland. It had been propagated for one to two generations from wild plants that had been collected within 25 km of the city of Zurich, except for *Agrostemma* which came from Brig (Canton Valais). In addition, we collected seed from one or two wild populations as controls. The wild seeds of *Papaver* were from populations in the cities of Zurich and Basle (F_1 only); those of *Silene* came from Buchs, 10 km west of Zurich. For *Silene* a wild-collected sib from New Jersey, USA, was used for producing an F_1 only.

CROSSING EXPERIMENTS

To make the hybrids, all plants were grown and crossed in a greenhouse. We crossed plants of foreign provenance with Swiss plants and made control crossings among the Swiss populations. All flowers were bagged with nylon mesh and hand-pollinated when the stigma was mature. In *Agrostemma* selfing within the flower was prevented by excising the anthers during the bud stage; this problem did not arise in either the strictly dioecious *Silene* or the wholly self-incompatible *Papaver* (M. Keller, unpublished data; cf. McNaughton & Harper 1964).

For the production of hybrids, five plants of *Agrostemma*, six of *Papaver* and eight *Silene* females were pollinated with a set of males from different origins (Fig. 1). Only one-way crosses were performed, always using the Swiss provenances as females; these were mated with the other sibs and with plants of the same stock as a control. Similarly, F_2 seed was produced by pollinating the same dams with pollen from F_1 progeny of previous crossings. A diverse set of F_2 hybrids was available for *Papaver*, whereas for *Silene* only Swiss × Hungarian F_2 hybrids, and for *Agrostemma* only Swiss × German hybrids (Hesse, central Germany), were produced. The number of different pollen donors within sibs varied due to the limited availability of some mature males, but for all hybrid types at least two different sires were used.
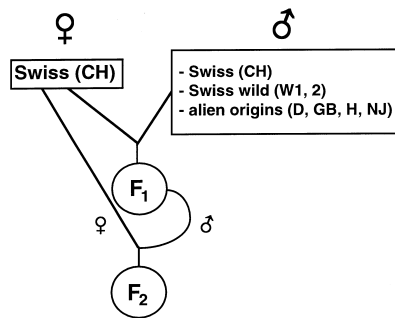
Fig. 1. Crossing design to produce hybrid lines. For F_1 progeny Swiss (local) plants of *Agrostemma*, *Papaver* and *Silene* were crossed with others from different origins. Only one-way crosses were performed, always using the Swiss provenances as females; these were mated with the other sibs, with plants of the same stock as a control, and with similarly produced F_1 for F_2 backcrosses. Abbreviations of the provenances: CH, Switzerland; D, Germany; GB, England; H, Hungary; NJ, New Jersey, USA; W, Swiss wild populations, Zurich (W1) and Basle (W2).

PLANT BIOMASS

Starting from 20 April 1998, the seeds were sown in a greenhouse in plastic trays with individual wells (5 cm diameter) for each plant. After 4 weeks the seedlings had reached a rosette diameter of about 12 cm and were transplanted into the field. The plants were arranged in randomized blocks of $2 \times 2 \, m^2$, the offspring of each dam being together in one block, with two replicates of each full-sib seed lot placed in either half-side (the blocks were not split into two for reasons of statistical design, but did ensure equal distribution of the two within-block replicates). The spacing between plants was 30 cm, which turned out to be rather dense for *Papaver* and *Silene*, and the plants were soon touching each other. For this reason the numbers of neighbouring plants were included in the analysis as a covariate to allow for possible competition effects. A thorough weeding was done about every 2 weeks. No grazing damage was observed and the plants were not protected against herbivores. A hailstorm caused some leaf damage soon after planting, mainly to the *Silene* rosettes, but the incident had no evident effect on further growth. Above-ground biomass of the plants was harvested at seed maturity (12–16 weeks in the field) and the dry mass was determined. In the case of *Silene* and *Papaver* the remaining seeds were shaken off before weighing; in the case of *Agrostemma* the seeds remained enclosed in the capsules on the plants (Firbank 1988).

A slightly different experimental design was used for *Agrostemma*, where the set of hybrids did not allow a balanced block design. In this species, two 8 $\times$ 7 blocks with completely randomized positions were set up in early May 1998 by sowing four seeds

in each position; an additional row of dummy plants (random genotypes) was planted on each side to avoid edge effects. After a 2-week period for germination, the excess seedlings were removed. All weeds were cleared from the experimental plots using a herbicide (glyphosate), the seedlings of *Agrostemma* being covered to protect them from the herbicide treatment.

SEED MASS

Seed mass was determined for *Agrostemma* and *Silene*. *Agrostemma* seeds were weighed individually, but the much smaller seeds of *Silene* were weighed in groups of 50. Of each seed lot from the *Silene* crossings, 50 seeds were taken and tested for viability under standardized conditions intended to produce maximal germination rates. Optimal conditions for germination of *Silene* were a 15-h light period at 25 °C and a 9-h dark period at 5 °C; 0·1% w/w gibberellic acid solution was added (Keller & Kollmann 1999).

DATA ANALYSIS

The biomass data were found to be normally distributed after square-root transformation (Shapiro–Wilk *W*-test). Above-ground biomass was used as the dependent variable within the ANOVA; missing or dead plants were excluded. In the case of *Papaver*, where such mortality was relatively high (17·2%), specific rates were compared (see below). The total numbers of harvested plants, mean biomass and survivorship in the field experiment are given in Table 1.

The ANOVA (type III sums of squares) was calculated on fixed effects, including introgression (main effect, standing for different hybrid types), mother (corresponding with block effect), father (nested in introgression), sex (*Silene* only), freedom and delay of germination. The latter two covariates proved to compensate well for different degrees of competition by adjacent plants and for delayed seedling growth after retarded germination; the variable sex contained the levels female (52% of individuals), male (41%) and vegetative (7%). Apart from father [introgression] in *Papaver*, all covariates contributed significantly to the model fit. Interaction terms were removed from the model after finding no significant interactions between all combinations of covariates.

Tests for statistical significance of heterotic and epistatic effects were performed by using contrasts at the crossing-type level within the ANOVA. For heterosis, the mean of both parents was compared with the F_1 offspring value (contrasts $\{0.5 \, P_1 + 0.5 \, P_2 = F_1\}$), whereas epistasis was investigated by relating the means of the parental and the F_1 values to that of the F_2 (contrasts $\{0.25 \, P_1 + 0.25 \, P_2 + 0.5 \, F_1 = F_2\}$; cf. Whitlock *et al.* 1995). As the F_2 had

Table 1. Life history and mating system of the study species, and performance in the common garden experiment as measured by above-ground biomass (mean ± 1SE) and mortality (*n* is sample size)

Study species	Life history	Mating system	Shoot dry weight (g)	Mortality (%)	*n*
Agrostemma githago	Annual crop weed	Selfing possible*	58·7 ± 4·2	2·7	112
Papaver rhoeas	Annual weed, ruderal	Self-incompatible†	32·6 ± 1·2	17·2	232
Silene alba	Perennial ruderal	Dioeceous‡	30·2 ± 1·1	4·4	248

*Firbank (1988).
†McNaughton & Harper (1964).
‡Baker (1947).

been produced by backcrossing instead of breeding within the F_1, their genomes consisted of 3/4 native and 1/4 alien parts. Therefore, the contrasts were set to $\{0.375\ P_1 + 0.125\ P_2 + 0.5\ F_1 = F_2\}$, which included an expectation of 50% heterosis as measured in the F_1. The chromosomal segregation (1 : 2 : 1) produces 50% F_2 offspring with chromosomes from different origin, while all F_1 plants ($= 100\%$) contain one chromosome of each parental line. The same weightings were used to calculate the deviation from the expected values, as shown in Figs 2–4. These weightings compensated for the ecological component of outbreeding, because the effect of foreign less adapted genes is given by the performance of alien parents and was included in the expected value according to the proportion of introduced foreign genes. Therefore, the deviation in the F_2 can be regarded as the outbreeding depression resulting only from epistasis, i.e. its physiological component. The mostly positive deviations within the F_1 were assigned to heterosis.

Survivorship rates in *Papaver* were compared with a GLM (generalized linear model) on the effects introgression, mother and father [introgression]. Because it was not possible to calculate IRLS (iteratively reweighted least-squares), common least-squares means were calculated for Fig. 5. The ANOVA model on seed mass and viability included the effects generation, mother and father [generation]. The GLM was calculated with the program S-Plus (1993), and all other analyses were performed with JMP® (SAS 1989–97).

Results

BIOMASS YIELD: HETEROSIS AND EPISTASIS

Above-ground biomass of almost all F_1 plants exceeded considerably the mean parental values (Figs 2–4). This result indicates that a heterosis effect was common among the first generation of hybrids.
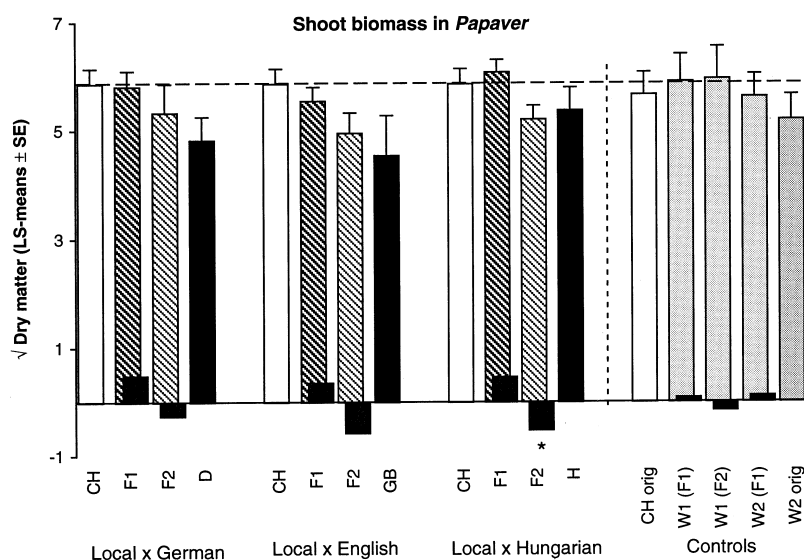

Fig. 2. Heterotic and epistatic effects on above-ground biomass of *Papaver rhoeas* lines. Results for Swiss and foreign parental plants, F_1 and F_2 backcrosses after one growing season; the degree of shading of the bars reflects the proportion of foreign genes. The small bars at the bottom line are deviations from the expected values and demonstrate heterosis (positive values) or epistasis (negative values). The dotted line marks the control level from crosses within the Swiss standard provenance (*$P < 0.05$). The control group on the right comprises the original local provenances (seed stock) and within hybrids. For abbreviation of provenances and crossing design see Fig. 1.
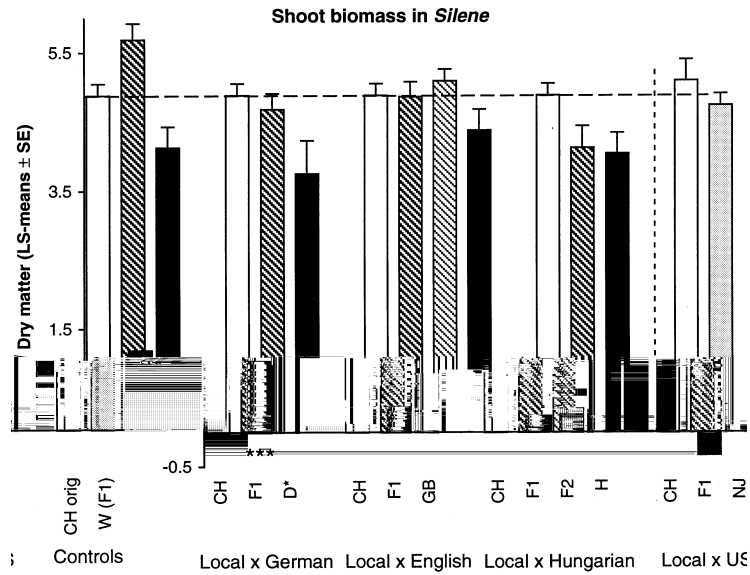
Fig. 3. Heterotic and epistatic effects on above-ground biomass of *Silene alba* lines. For details see caption to Fig. 2 (D*, hybrid between *Silene latifolia* ssp. *latifolia* and *S. latifolia* ssp. *alba*; other abbreviations of provenances and crossing design as in Fig. 1; ****P* < 0·001).

The highest heterosis was for a *Silene* hybrid, and resulted from crossing with a different subspecies, i.e. *Silene latifolia* ssp. *latifolia* from Bavaria, Germany. In contrast, only slight heterosis effects (1–3%) occurred in the two crossings among Swiss *Papaver* populations. The Swiss × US (New Jersey) F_1 hybrid of *Silene* exhibited negative heterosis. This so-called 'outbreeding depression' sometimes occurs even in the first hybrid generation, when the genetic distance between parental populations is high (cf. Emlen 1991).

The F_2 backcrosses deviated in most cases negatively from the expected values (Figs 2–4), with the exception of the Swiss × Hungarian F_2 of *Silene*. Although all other values showed the same negative trend, statistical significance was only reached for the Swiss × Hungarian *Papaver* backcrosses (ANOVA: $F_{1,40} = 4·17$, $P = 0·043$) and when all alien F_2 were pooled in the analysis. The control F_2 backcrosses between two different Swiss sources of *Papaver* revealed virtually no reduction in above-ground biomass. The strongest outbreeding depression of 15% loss was observed in F_2 plants of *Agrostemma* (ANOVA: $F_{1,48} = 5·84$, $P = 0·018$; Fig. 4).

The expected environmental component of outbreeding depression can be estimated directly from differences in the growth performance of Swiss and alien parental plants (Figs 2–4). All alien parents of *Papaver* and *Silene*, including the distant (70 km) Swiss *Papaver*, produced markedly less biomass than the two local provenances. However, the *Agrostemma* from central Germany performed as well as the Swiss provenance.
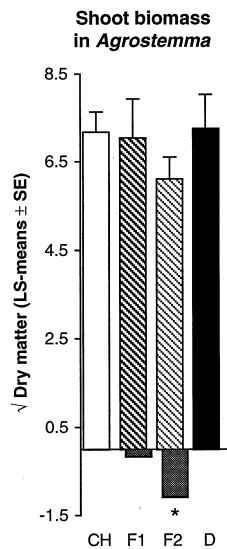
SURVIVORSHIP

The mortality of *Papaver* during the experiment was slightly higher in both the F_1 (10·5%) and the F_2 (11·5%) than in the control (within-local crossings, 4·2%) and the local parent (0%). Survival values with regard to the covariables mother(block) and father are shown in Fig. 5. The degree of competition and delayed germination had no effect on survi-
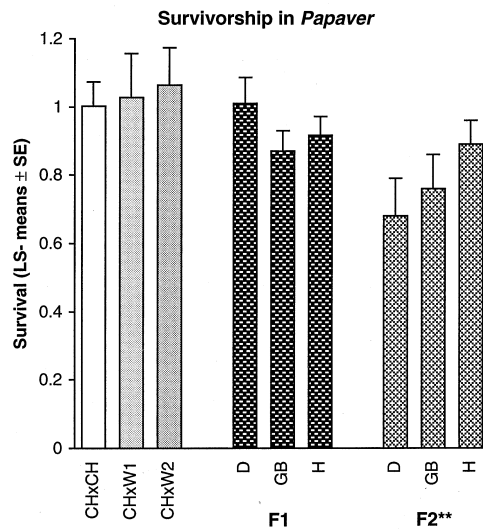

Fig. 4. Shoot biomass of *Agrostemma githago* parents and the hybrid line from crossings with a population from Hesse, central Germany. The bars at the bottom line are deviations from the expected values (**P* < 0·05). Abbreviation of provenances and crossing design as in Fig. 1.

Fig. 5. Survivorship of *Papaver* crosses in the field experiment. Common least-squares means were calculated with a model including the covariables mother, father and freedom. Significance ($P < 0.01$) is only given between pooled controls and F_2 values, as tested with a generalized linear model. The F_1 and F_2 are crosses of Swiss local plants with plants from Germany, England and Hungary (for abbreviation of provenances and crossing design, see Fig. 1).

val. The GLM analysis had low statistical power and revealed significant differences only for pooled controls and F_2 plants (nominal logistic model: $\chi^2 = 4.00$, $P = 0.046$), but the downward trend of the hybrids with distant introgressions was obvious. The odds-ratio of 2.03 indicated that the probability of a non-viable individual belonging to the F_2 was twice as high, while it was equal for the control to F_1 (odds-ratio 0.97).

SEED MASS

The data for seed mass of outcrossed *Agrostemma* and *Silene* hybrids revealed similar trends as for above-ground biomass, with a positive deviation in the F_1 and a negative effect in the F_2 backcrosses (Figs 6 and 7). While there was only a slight tendency in *Agrostemma*, the seed weight in *Silene* was significantly higher in F_1 hybrids (ANOVA: $F_{1,32} = 6.89$, $P = 0.011$) and was lower in the F_2 backcrosses (ANOVA: $F_{1,69} = 9.49$, $P = 0.003$). It is interesting that germination tests revealed no differences in seed viability amongst any of the *Silene* crossings ($> 99\%$ in both parents and offspring; M. Keller, unpublished data).

Discussion

OUTBREEDING DEPRESSION

Although statistical significance was not always achieved, our data revealed a clear tendency towards outbreeding depression due to epistasis in *Papaver*, expressed both by reduced biomass and viability (survival) of the F_2 backcrosses. The even stronger

negative effect in the hybrid progeny of *Agrostemma* supports the hypothesis that inbreeding species are more sensitive to introgression from distant populations. The lack of an epistatic effect in the Swiss × Hungarian F_2 of *Silene* is unexpected; for any herbaceous species we would expect some decrease in the fitness of F_2 plants when the parental populations are separated by a distance of about 1000 km.
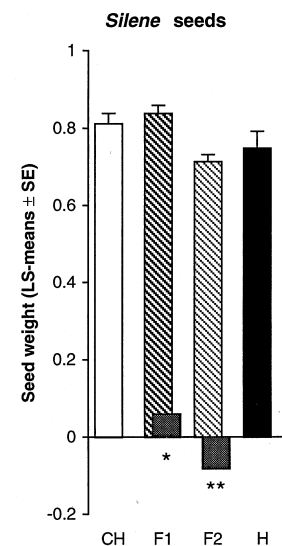

Fig. 6. Seed mass of parents and hybrids from long-distance crosses of *Silene alba* under experimental conditions. The small bars at the bottom line give deviations from expected values. The least-squares means compensate for maternal, paternal and plot variability (*$P < 0.05$; **$P < 0.01$). The F_1 and F_2 are crosses of Swiss local plants with plants from Hungary (for abbreviation of provenances and crossing design, see Fig. 1).
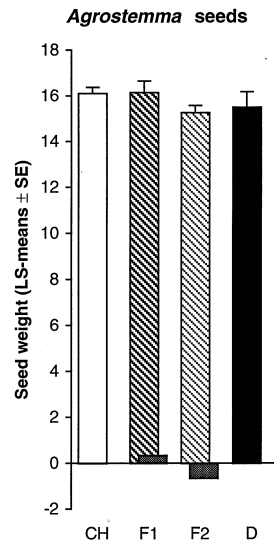
Agrostemma seeds

Fig. 7. Seed mass of parents and hybrids from long-distance crosses of *Agrostemma githago* under experimental conditions. The bars at the bottom line give deviations from expected values. The least-squares means compensate for maternal, paternal and plot variability. The F_1 and F_2 are crosses of Swiss local plants with plants from Germany (for abbreviation of provenances and crossing design, see Fig. 1).

environmental influences (Antonovics & Schmitt 1986). However, absolute seed mass of different ecotypes or provenances can hardly be regarded as a measure of fitness. Only within a population could smaller seeds signify reduced fitness and then only if they lead to lower seedling performance. In fact, no effect of seed mass on germination success and survivorship was detected in *Anthoxanthum odoratum* (Schmitt & Antonovics 1986), although in other species such effects have been demonstrated (Harper & Obeid 1967).

The uniformly high seed viability of *Silene* in the germination tests highlights the important difference between testing under optimal and natural conditions. While our assay in light chambers produced practically complete germination, the performance under natural conditions might have differed amongst the various provenances because of specific adaptations to their home conditions.

ECOLOGICAL VS. PHYSIOLOGICAL OUTBREEDING DEPRESSION

In some species, the fitness of immigrants can be less than half that of local plants (Levin 1984), although such drastic differences have been found chiefly between populations from strongly contrasting habitats. Relative reductions in fitness of 8–23% appear to be more common, as we observed in the foreign provenances of *Papaver* and *Silene*. Plants from local populations were always more productive than foreign parents, including the distant Swiss *Papaver*, but with the exception of the *Agrostemma* from central Germany (Fig. 4). This advantage of the local populations is consistent with results on plant establishment from a previous field experiment at two arable sites, where the local plants of six out of eight species outperformed those from other countries (Keller & Kollmann 1998). The lack of environmental maladaptation in *Agrostemma* might reflect the
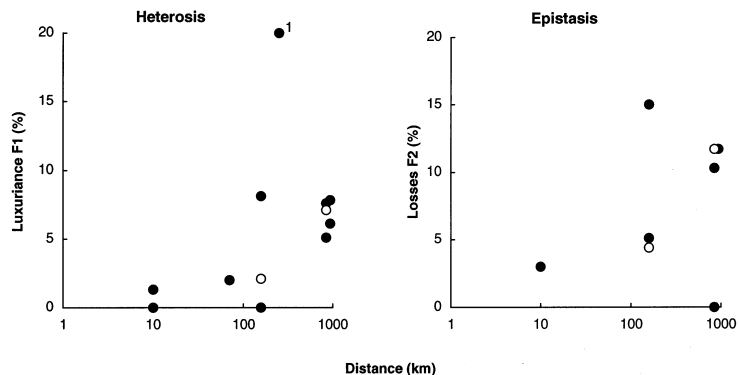
On the other hand, the high potential for gene flow in *Silene* (pollen transport over 640 m has been reported; McCauley, Raveill & Antonovics 1995) and its strong colonizing ability, which is probably enhanced by human activity, may mean that this species is less subject to outbreeding depression than many others.

The seed mass from plants with alien introgressions was affected in the F_2 backcrosses of both *Silene* and *Agrostemma*. The differences are surprisingly high, as paternal effects on seed size are normally dwarfed by the more important maternal and

Fig. 8. Heterosis ('luxuriance', positive deviations from expected values in F_1) and epistasis ('losses', negative deviations in F_2) plotted against grossly estimated geographical distance on a log scale. The values are from all three species and both above-ground biomass (filled symbols) and seed mass (empty symbols) measurements (1, hybrid between two subspecies of *Silene latifolia*).

special character of crop mimics (Thompson 1973), where close adaptation to agricultural management is probably more important than to local environmental conditions.

The decline in fitness due to poor environmental adaptation after introgression exceeded the effect of intrinsic co-adaptation in the F_2 (see values of alien parents in Figs 2 and 3). Nevertheless, the first component of outbreeding depression is expected to recover with each subsequent crossing within the local population, because of thinning of the alien genes and selection against unfavourable genes. In contrast, the impact of the second component may increase, at least for some generations. This expectation is based on the assumption that there is progressive disintegration of gene complexes with increasing recombination (Wallace 1955; Lynch 1991). These contrasting trends lead to the tentative suggestion that, in considering the fate of natural populations, the epistatic component is more important than the loss of optimal adaptation, because the re-establishment of parental or new, comparably successful, recombinants will take more time. This, however, is speculation and needs further investigation.

If losses in fitness due to disturbed gene interactions do increase in subsequent hybrid generations, negative consequences for populations after such introgressions could be serious, especially in the case of repeated introgression from introduced wildflowers and where natural populations are small. Among the European arable weeds, there are many highly endangered species with critically small and highly fragmented populations, e.g. *Adonis aestivalis*, *Bupleurum rotundifolium* and *Delphinium consolida* (Landolt 1991). However, it is difficult to predict whether, in the long term, such local populations will experience substantial genetic disturbance or whether they can recover after a few generations. According to Templeton (1986), this depends on the degree of integration of the genome: 'If co-adaptation involved nearly all the genes of an organism, there would be virtually no chance of re-establishing a parental genotype, and the time needed to select a fit recombinant would be very long'. If, on the other hand, the number of loci responsible for the relevant fitness interactions is small, outbreeding depression may only be a temporary phenomenon (Templeton 1986). Data from population studies so far indicate that in this respect plants are very variable, so that general conclusions on the intensity and importance of intrinsic co-adaptation cannot be made (Roff 1998).

Because the negative effects of mixing increase rapidly with increasing distance between populations, it seems reasonable to suppose that the transport of seed material over hundreds of kilometres, and the associated genetic exchange, could destabilize local conspecific populations. According to Ell-

strand & Elam (1993) 'gene flow rates of 1–5% are sufficient to counterbalance selection for local adaptation'. Furthermore, and in contrast to earlier expectations, high gene flow rates in weeds (*Raphanus raphanistrum*) have been observed over thousands of metres from large sources (as are wildflower strips) into small stands (Ellstrand, Devlin & Marshall 1989). On the other hand, selection coefficients can be > 5% in the presence of strong selection factors, and in this case will produce local adaptation even in the face of gene flow (Jain & Bradshaw 1966; Slatkin 1973; May, Endler & McMurtrie 1975)

EFFECTS ON GENETIC DIVERSITY

Besides genetic integrity, the large-scale use of seed from a few sources presents a potential threat to biodiversity at the microevolutionary scale. For programmes of restocking or agricultural landscaping, large amounts of plant material are produced from one or a few stands and used over large areas. This rather uniform genetic material can reduce genetic diversity and interfere with the genetic structure of locally differentiated populations. While this may affect many species, those with small fragmented populations probably experience the most extreme form of this process, i.e. genetic assimilation. Therefore, small populations face not only problems of inbreeding, genetic drift and genetic melt-down (Gilpin & Soulé 1986; Lynch, Conery & Bürger 1995; Fischer & Matthies 1998), but are vulnerable to genetic assimilation under large-scale introductions of alien plants, i.e. the original genes are swamped out by hybridization with a larger introgression source (Ratcliffe 1973). Although the term 'genetic assimilation' has been used for discrete taxonomic units, such as subspecies or ecotypes, the same process may work within small locally adapted populations. This would result in a loss of 'endemic' alleles and the dominance of the introduced genotypes over a large area of the agricultural landscape. This topic has been discussed intensively by many authors including Daily & Ehrlich (1995), Millar & Libby (1991) and Guerrant (1996).

SUPPORTING DECLINING POPULATIONS BY PLANTING RARE SPECIES?

The endangered state of many plant species has been caused by population losses, most commonly brought about by human activities. For this reason, some conservationists have seen the need to support weak populations by translocating seed or transplants (van Groenendael, Ouborg & Hendriks 1998). However, whether it is possible to overcome the 'extinction vortex' (Gilpin & Soulé 1986) by introducing foreign plant material remains a matter of debate (Hodder & Bullock 1997). Fenster &

Dudash (1994) even deny a 'natural basis' for preserving the genetic integrity of a species, and suggest that the break-up of gene complexes by mixing distant sources may be a chance for enhanced environmental adaptation. Indeed, 'gene flow management' has been suggested as a measure to invigorate endangered populations that are threatened by genetic erosion and inbreeding depression (Ellstrand 1992; Ellstrand & Elam 1993; Pegtel 1998). We agree with these authors on the need for a careful evaluation of the particular circumstance of a species before undertaking such measures. A successful example of gene flow management in animals was reported by Westemeier *et al.* (1998) for remnant populations of greater prairie chickens (*Tympanuchus cupido* ssp. *pinnatus*).

Part of the problem is that although offspring fitness has often been observed to increase after inter-populational crosses, most studies on outbreeding effects have examined only the F$_1$ generation (Fenster 1991; Bijlsma, Ouborg & van Treuren 1994; Waser & Price 1994; Oostermeijer, Altenburg & den Nijs 1995). Using such results to extrapolate to the future of the populations in question can be misleading, because epistatic outbreeding depression is ignored. Many experiments, including ours, show that the F$_1$ often performs as well as the local parent or better, but the benefit by heterosis has to be 'paid for by a segregational genetic load' (MacKey 1976), meaning that later generations may suffer from reduced populational stability. However, the results of our crosses between native and foreign populations cannot be applied directly to rare species, where the material for genetical re-enforcement would probably come from the geographically closest viable populations (van Groenendael, Ouborg & Hendriks 1998).

That genetic variation should be as high as possible (van Andel 1998) has been suggested as a principle for species reintroductions, but it should not be applied uncritically within declining populations, as also emphasized by Fischer & Matthies (1997). However, Fenster & Galloway (in press) conclude from an extensive study that restoring populations using genotypes from distant sites will have no deleterious consequences if local adaptation is limited. Moreover, findings such as those of Tecic *et al.* (1998) suggest that the risks of population extinction due to outbreeding depression in some species, at least, may be much smaller than those due to inbreeding and environmental stochasticity.

GUIDELINES FOR SEED SOURCE DISTANCES?

The geographical distances relevant in this context do not necessarily correspond with genetic distances, although on the large geographical scale there is often a good correlation (Fenster & Dudash 1994;

Knapp & Rice 1998). Defining source areas of plant material for introduction is difficult, as local adaptation may occur even at a scale of a few hectares (Argyres & Schmitt 1991; Waser & Price 1994) and there may even be local adaptation within continuous populations (Waser 1993). Fischer & Matthies (1997) found significant outbreeding depression in small isolated populations of *Gentianella germanica* 25 km apart. However, more abundant species with stable populations are probably less sensitive, and an experimentally measured small outbreeding depression is not necessarily significant for a population in a natural setting. It is unrealistic to prescribe a maximum distance for plant introduction because, for most species, it is unknown where the outbreeding effect begins to become severe. Certainly, there is no general translocation distance that can be regarded as safe for most plant species.

Although negative outbreeding effects have been observed at distances of only a few kilometres, such short-distance transport probably poses little threat to common ruderal plants and arable weeds. In some regions, however, introgression over some 100 km could cause fitness losses similar in degree to those observed when Swiss plants were crossed with plants of English and Hungarian provenance in the present study (Fig. 8). For countries where large quantities of seed of wild plants are produced and dispersed, it seems reasonable to develop guidelines about seed source as well as appropriate control procedures. Such guidelines may vary specifically according to the regional geographical properties. Switzerland, for example, is structured in distinct phytogeographic regions, reflecting climatic and edaphic differences (Landolt 1991). For species that are neither rare nor differentiated into subspecies or ecotypes, it seems sensible to decide on the basis of such phytogeographical regions what is an acceptable 'distance' over which plants can be introduced. For example, the Swiss Midlands form a moderately uniform phytogeographical unit of about 300×50 km^2. For this region guidelines were proposed to regulate the use of seed of wild plants in the agricultural landscape (Keller 1999). However, much more research is needed for many plant species before more general rules can be formulated.

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