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Dynamic management of genetic resources: maintenance of outcrossing in experimental metapopulations of a predominantly inbreeding species

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Abstract

Dynamic management of genetic resources in predominantly inbreeding species requires increased levels of outcrossing to limit the loss of genetic variation due to smaller effective sizes and to favour the emergence of new genetic combinations. Here, we show that outcrossing rates can artificially be permanently increased in experimental evolving plant metapopulations, using *Arabidopsis thaliana* as a model. We introduced male-sterility genes and used an adequate management of the resulting female plants to modify the outcrossing rates. As expected, the increase in outcrossing resulted in lower levels of heterozygote deficiency (F_{is}) than observed in natural populations of *A. thaliana* and therefore in the maintenance of potentially higher levels of genetic variation. An additional selective advantage for females' offspring, due to the production of larger seeds by females and a possible heterosis effect, furthermore led to smaller F_{is} than expected from the realized outcrossing rate. This selective advantage also resulted in an increase in female frequency, especially in metapopulations with large population sizes, creating a non-causal negative correlation between female frequency and heterozygote deficiency.

Introduction

Conservation policies aim at preserving the ability of populations to respond to natural or artificial selection. Conservation methods are therefore required that preserve genetic variation for selected traits, but also currently neutral genetic variation, a potential target of selection in case of environmental changes (Frankham 1999). In this respect, dynamic management of genetic resources, where genetically heterogeneous populations are grown in different environments, should play a central role in conservation genetics, especially for crop species whose genetic diversity is for the moment mainly conserved in gene banks. A dynamic management scheme aims at maintaining the level of genetic variation of the conserved populations, but it also allows the populations to respond to changes in their biotic or abiotic environment (Allard 1992; Goldringer et al. 2001; Henry et al. 1991; Kahler et al. 1975; Long et al. 2000). The pool of genetic diversity is not static and the emergence of new beneficial genetic combinations, or, in the long term, even new mutations, can compensate for the inevitable loss of alleles.

The efficacy of such dynamic management depends on key parameters of the conserved populations. The mating system, which influences both the dynamics of adaptation (see e.g., Bürger 1999) and the maintenance of genetic variation, is one such key parameter. The levels of neutral and selected genetic diversity in a population are theoretically negatively correlated with the selfing rate. For example, heterozygosity at neutral loci is expected to be smaller in more inbred populations (Wright 1969), leading to lower effective population sizes and lower effective recombination rates (Pollak 1987), the latter resulting in a strengthening of selective sweep and background

selection effects (see Charlesworth and Charlesworth 1995). Similarly, quantitative genetic variation under selection is also expected to decrease with decreasing outcrossing rates, regardless of the mechanisms maintaining variation (overdominance or mutation-selection balance, Charlesworth and Charlesworth 1995). These expected negative correlations between the selfing rate and the level of genetic variation were indeed observed in natural populations, most clearly for allozymic variation (Hamrick and Godt 1996; Schoen and Brown 1991) and nucleotide diversity (Liu et al. 1999, 1998), but also, to a lesser extent, for quantitative variation (Charlesworth and Charlesworth 1995). Besides the maintenance of higher levels of genetic variation, outcrossing prevents the accumulation of mildly deleterious mutations, and small, isolated populations of predominantly selfing species are expected to be doomed to extinction, due to random genetic drift and mutation accumulation (Schultz and Lynch 1997).

In order to maximize the amount of conserved genetic variation and to maintain populations in the long term, it thus appears crucial to have a comprehensive knowledge of the mating system of managed populations, and, ideally, to be able to artificially decrease the selfing rate for predominantly selfing species (Enjalbert et al. 1998). Plant breeders rely on two main genetic systems, self-incompatibility and male-sterility, to produce hybrids in self-pollinating species (e.g., Dattée et al. 1992); they could be used as a means to manipulate the breeding system during dynamic management. However, their transfer to managed evolving populations is not straightforward: when outcrossing is maintained via self-incompatibility loci, the realized selfing rate in a population strongly depends on the number of loci and alleles involved, and is not directly accessible to the manager. Male-sterility seems at first preferable, because cross-pollination occurs mostly on female plants that are generally easily identified. However, the maintenance of both females and hermaphrodites over time requires a more than two-fold fecundity advantage for females or large inbreeding depression in the case of nuclear male-sterility. A smaller female advantage but also more complex mechanisms, including costs for nuclear restorers of male-fertility, are required for the maintenance of two sexes in the case of nucleo-cytoplasmic male-sterility (Gouyon and Couvet 1987).

In this paper, we develop a model study to consider achievability and relevance of increased outcrossing in managed populations. We present results from 10 generations of an experimental dynamic management in metapopulations of the usually predominantly selfing species, *Arabidopsis thaliana*. Using nuclear male-sterility, we attempted to increase the level of outcrossing in these managed populations. We recorded the change in female frequency and the level of heterozygote deficiency in metapopulations of various population sizes. We place our results in the context of conservation of genetic resources of crop species and discuss the applicability of increasing outcrossing rates in dynamic management of wild species.

Methods

Experimental set-up

In order to assess the optimal conditions for a dynamic management of genetic resources, six metapopulations of *Arabidopsis thaliana* were grown in the greenhouse for 10 generations. These metapopulations comprised 20 populations each, and differed by local population size (10, 25 or 100 individuals per population, two replicate metapopulations per population size treatment).

A. thaliana is a predominantly selfing species, with selfing rates around 98–99% in natural populations (Snape and Lawrence 1971). We introduced a nuclear recessive allele conferring male-sterility into the initial metapopulations to increase outcrossing. The parental lines used for founding the metapopulations therefore comprised a male-sterile mutant (nw77 mutant, *pistillata*, Nottingham Arabidopsis Stock Centre) and 14 lines of *A. thaliana* collected from nine natural populations in France and the United Kingdom (Table 1). These 15 parents were control-crossed following the protocol described in Table 1; the F1 generation was selfed and the resulting F2 seeds were pooled according to the proportions given in Table 1. This pool of seeds was used to sow the first generation of each population. The initial metapopulations were thus all genetically similar, except for sampling variability, with an expected heterozygote deficiency of 0.45 (calculated from the initial frequencies of each parent). The initial frequencies of the allele conferring male-sterility and of the male-sterile plants were 12.5% and 6.25% respectively. Male-sterile individuals are functional females (no stamens are produced) and will therefore be named “females” from now on.

Table 1. Crosses at the origin of the F2 and their contribution to the first generation

Cross	Contribution	Cross	Contribution
$GB_1 \times F78b$	10%	$F45 \times F28_2$	10%
$(GB_1 \times F06_1) \times NW77$	10%	$(F45 \times F06_3) \times NW77$	15%
$GB_1 \times F28_1$	5%	$F37 \times F06_4$	5%
$F37 \times F28_2$	10%	$F22 \times F28_1$	10%
$F69 \times F06_2$	5%	$GB_2 \times F78a$	20%

GB = Great Britain, F = France. For French populations, numbers indicate the district where the population was collected, and letters are used when two populations are collected in the same district. Numbers in subscript differentiate individuals coming from the same population.

Each generation, seeds were sown following a regular grid, watered with a solution containing 0.15% fungicide (Dericlor, Ciba Geigy) and left for one week in the dark at 4 °C to break dormancy. Density was constant across population sizes using different pot sizes: 26.4 cm^2 (10 plants), 86.25 cm^2 (25 plants) and 350 cm^2 (100 plants), one population per pot. After germination, the plants were grown in a climate-controlled compartment of a greenhouse under a 16 h light / 8 h dark photoperiod, 15 °C night and 20 °C day, and were watered twice a week. Female plants were managed as follows: each generation, twice or three times during flowering, flowers of female individuals were gently rubbed with the maximum number of male-fertile flowers of the population to ensure seed production. The newly sown populations consisted on average of 10% seeds harvested at random from females within the population, 88% seeds harvested from hermaphrodites and 2% migrant seeds coming from another randomly chosen population within the metapopulation (migrant pool model). The number of migrants was drawn at random from a Poisson distribution with mean Nm for each population, where N stands for population size and m for migration rate. Among the migrant seeds, 10% came from females and 90% from hermaphrodites on average. The outcrossing rate therefore equalled 10.2% plus the small amount (1–2%, Snape and Lawrence 1971) of uncontrolled crossing. It did not depend on female frequency, provided that at least one female was present in each population (otherwise, the outcrossing rate fell to the usual 1–2% in this population).

Change in female frequency

Over ten generations, the frequency of female plants was scored at harvest each generation (except generation 1, not scored), for each population in the six metapopulations.

We calculated the expected equilibrium female frequency in this specific experimental set-up as follows. Because females and hermaphrodites' contributions to the following generation were kept independent on their respective frequencies, the experimental design induced a selection on females. For example, when females were rare in a population (e.g., 1%), their contribution to the next generation (10.2%) was much larger than would be expected under strict neutrality (1%). On the contrary, frequent females (> 10.2%) were counterselected. The relative female advantage f (i.e., the ratio of individual female seed contribution to the following generation to hermaphrodite seed contribution) therefore depended on female frequency (x) and could be calculated as follows. S_g, the mean individual seed contribution of gender g, is the ratio of its total seed contribution in one population (including immigrants) to its frequency in this population. The progenies of females represent 10% of a new population plus 10% of immigrant seeds: $S_f = 0.1\ (1 + m)\ N / x$ and the progenies of hermaphrodites represent 88% of a new population plus 90% of immigrant seeds: $S_h = (0.88 + 0.9m)\ N / (1 - x)$, where N stands for population size and m for migration rate ($m = 0.02$).

Since f is the ratio of female seed contribution to hermaphrodite seed contribution,

$$f = [0.102/x]/[0.898/(1 - x)] \qquad (1)$$

In addition, the expected equilibrium female frequency (x) in a population consisting of females and hermaphrodites is:

$$x = (f - 2)/2(f - 1) \qquad (2)$$

(Lewis 1941) whatever the mating system of hermaphrodites (Gouyon 1983). Solving equations (1) and (2) leads to an expected female frequency of 0.05 at equilibrium. Recurrence equations indicate that

female frequency is expected to reach the equilibrium value after two or three generations (see appendix). We tested whether the metapopulation means differed from the expected equilibrium frequency from generation 2, with a Student test.

Indirect selective advantage for females

Male-sterility was maintained through artificially induced female advantage as explained above, but this does not preclude a possible simultaneous "natural" advantage for females or for their progeny. The coexistence of females and hermaphrodites in a population is generally explained by two non-exclusive mechanisms: (1) greater fecundity of females, due to enhanced seed-set or better quality of seeds via resource reallocation (e.g., Darwin 1877) and (2) selective advantage for females' offspring, due to heterosis or maternal effects (e.g., Charlesworth and Charlesworth 1978). A larger seed-set of females can immediately be excluded because the experimental set-up fixed the number of seeds from females in the following generation. Two remaining mechanisms, seed-size effect and heterosis, affecting offspring of females only, were tested in the experimental metapopulations.

Quality of females' progeny due to seed size

Females produced fewer seeds than hermaphrodites, because hand-pollination was not very efficient. These seeds were therefore larger than seeds from hermaphrodites, most probably due to resource reallocation: the mean (± SE) seed length in pooled females' progenies was 0.56 ± 0.05 mm (N = 100 seeds), and the mean (± SE) seed length in pooled hermaphrodites' progeny was 0.46 ± 0.04 mm (N = 100 seeds). The difference was highly significant ($t = -14$, $df = 198$, $P < 0.0001$).

We tested whether plants growing from larger seeds were fitter in terms of fruit production, by sampling small and large seeds in various progenies of hermaphrodites, and by growing them in competitive conditions. We used only seeds from hermaphroditic mothers to avoid the possible confounding effect of heterosis. In each metapopulation with 100 plants per population, nine hermaphrodites coming from six different populations were selfed. In each of the 18 resulting progenies, 30 seeds were sampled and classified into 15 "small" and 15 "large" seeds. The mean (± SE) seed lengths were 0.38 ± 0.04 mm and 0.52 ± 0.04 mm for small and large seeds respectively, these measures being conducted on a subsample of 10 seeds per progeny. Each progeny was sown in a separate pot; seeds were sown on regular grids (approximately 1.5 cm between each seed), such that each seed had three large and three small neighbour seeds. To prevent border effects, border plants were eliminated from analyses. Plants were collected at the end of their life cycle, and number of fruits was scored. Plants that died during the experiment were included in the data set, with zero fruit production. Some female plants were observed in three progenies, because the selfed parent was heterozygous at the male-sterility locus. These female plants were excluded from the data set because female fruit production is always low, even if they are hand-pollinated. The results of the statistical analyses were the same whether the families containing females were included or not (data not shown).

Fruit number was here used as an indicator of plant fitness, because (1) selfing was predominant (in that specific experiment), (2) fruit number is generally not negatively correlated with seed number per fruit in *A. thaliana* (Andalo et al. 2001) and (3) germination rates are high (> 97% in this experiment). Seed size effect was tested using a mixed model ANOVA (SAS Institute 2000) on fruit number, with the following model:

$$Y_{ijk} = \mu + Prog_i + size_j + (Prog \times size)_{ij} + R_{ijk}$$

with $i = 1 \ldots 18$ and $j = 1, 2$. Progeny (*Prog*) and (*Prog* × *size*) were regarded as random effects. The distribution of the ANOVA residuals slightly deviated from normality, fruit number was however left untransformed because no usual transformation improved normality, and ANOVA is known to be robust to non-normality.

Quality of progeny due to heterosis

Heterosis is unlikely to be strong in natural populations of *A. thaliana*, because inbreeding depression is infrequent in populations of predominantly selfing species (Husband and Schemske 1996). However, the experimental metapopulations originated from crosses between plants from geographically and genetically distant populations, where different deleterious mutations have probably been fixed. These crosses may have created genetic variation for deleterious mutations in the initial metapopulations, and, as a consequence, heterosis. We compared the progeny of crosses between more or less inbred parents to

detect this possible heterosis. Only female plants were used as female parents because crosses are not easy to perform on hermaphrodites. Two hermaphroditic plants, coming from a metapopulation with 100 plants per population, and that were heterozygous at the male sterility locus (MSms), were selfed, which provided two families containing approximately $\frac{1}{4}$ females and $\frac{3}{4}$ hermaphrodites. In each family, five females were randomly chosen and were crossed with eight different hermaphrodites, corresponding to four crossing types: two full-sibs (FS), two plants from the same population (POP), two plants from another population within the metapopulation (MPOP), and two "external" plants coming from a German population (EXT). Each cross was performed on a distinct branch of the female plants, and the spatial distribution of crosses on a female depended on branch availability when the various fathers flowered: this resulted in no pattern in the spatial distribution. The seeds resulting from the eight crosses were harvested separately. Seeds from a same female parent were sown on a regular grid, in competitive conditions. For each crossing type, 16 seeds were sown, and the 128 seeds (= 16 seeds × 8 crosses) were randomly distributed in a pot. To prevent border effect, the border row was eliminated from statistical analyses. As in the preceding experiment, plants were collected at the end of their cycle, and number of fruits was scored. Plants that died during the experiment were included in the data set with a zero fruit production. Female plants were excluded, as described above.

The effect of the crossing type was analysed with a nested ANOVA on fruit number, with the model:

$$\begin{aligned} Y_{\mathrm{ijklm}} \;=\; & \mu + cross_{\mathrm{i}} + Fam_{\mathrm{j}} + Mother(Fam)_{\mathrm{k}} + \\ & Father(cross \times Fam)_{\mathrm{l}} + (cross \times Fam)_{\mathrm{ij}} + \\ & (cross \times Mother(Fam))_{\mathrm{ik}} + ((Mother \times \\ & Father)(cross \times Fam))_{\mathrm{ijkl}} + R_{\mathrm{ijklm}} \end{aligned}$$

where the effects are abbreviated as follows: *cross* = cross type ($i = 1 \ldots 4$), *Fam* = family ($j = 1, 2$), *Mother* = female parent plant ($k = 1 \ldots 5$) and *Father* = male parent plant ($l = 1, 2$). *Fam*, *Mother*, *Father* and their interactions were regarded as random effects. The *cross* type effect was tested using the *Father* mean square as the error and the *Family* effect was tested using the *Mother* mean square as the error. The distribution of the ANOVA residuals slightly deviated from normality, but fruit number was left untransformed, because no usual transformation improved normality and ANOVA is known to be robust to non-normality.

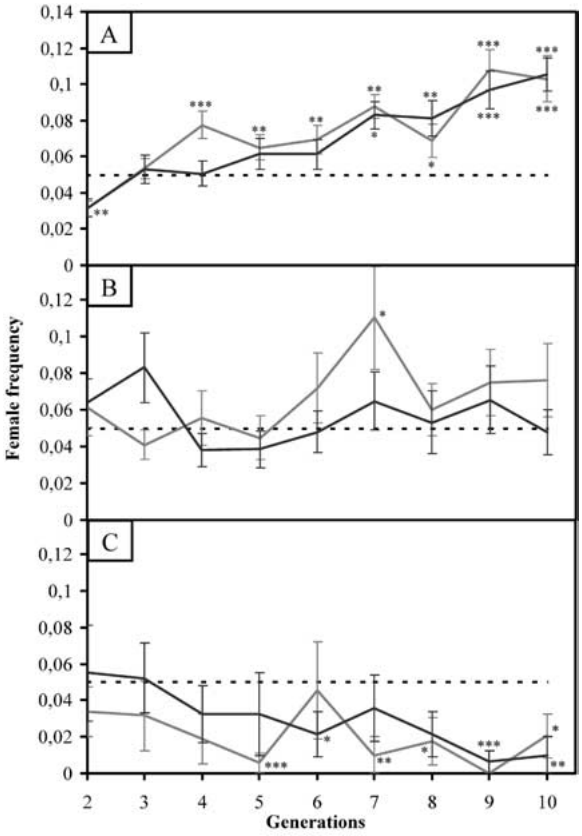

Figure 1. Change in female frequency over 10 generations in the two replicate metapopulations with 100 (A), 25 (B) and 10 (C) individuals per population (solid lines). Means and standard errors were calculated over the 20 populations of a metapopulation. Dotted lines (- - -) represent the expected equilibrium (5%) under the assumption of no natural female advantage, and stars (∗) indicate a significant difference between the observed and this expected frequency, tested with a Student test. Levels of significance are: $^{*}0.01 < P < 0.05$, $^{**}0.001 < P < 0.01$ and $^{***}P < 0.001$.

Change in mean heterozygote deficiency

The mean heterozygote deficiency F_{is} was estimated at generations 4, 7 and 10, for each metapopulation, using four isozyme loci (see Lavigne et al. 2001 for details of the methods) and the *Fstat* software (Goudet 1995).

Results

Change in mean female frequency: The change in female frequency depended on population size. At large population size (Figure 1A), female frequency increased in both metapopulations, and, after generation 6, significantly exceeded the expected equilib-

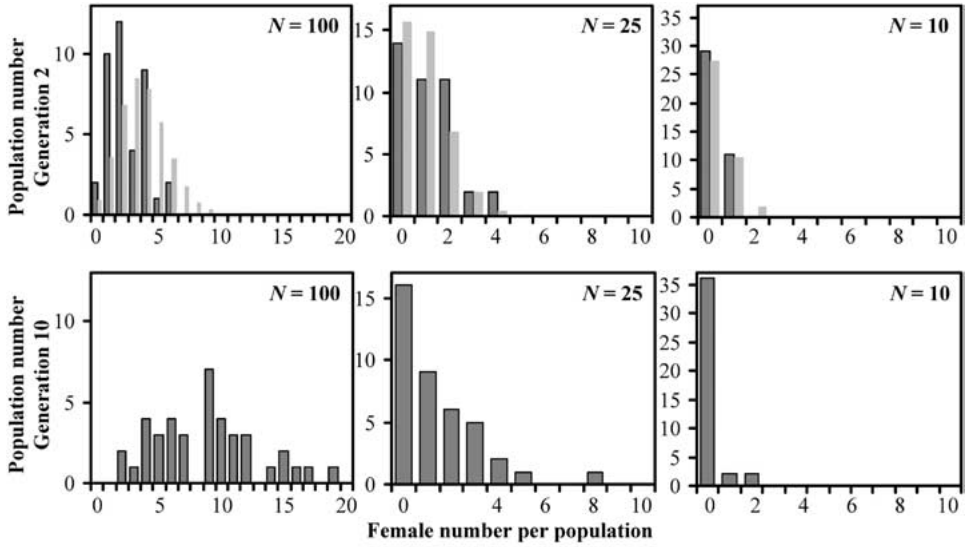

Figure 2. Distribution of expected (light grey) and observed (dark grey) female number per population. The expected distribution is a Binomial with parameters N and 0.037. The observed distributions are plotted for the 40 populations (2 metapopulations × 20 populations) of a same population size treatment, for each population size and at generation 2 and 10. The scales of the x and y-axis differ across population size treatments.

rium frequency (0.05) for both metapopulations. On the contrary, at small population size (Figure 1C), female frequency continually decreased and females were nearly extinct at generation 10 (female frequency significantly smaller than 0.05 for both metapopulations). For the intermediate population size (Figure 1B), the female frequency remained globally constant around the theoretical expectation (only one significant difference over the 10 generations).

Distribution of female frequency among populations: In the second generation the number of females in one population theoretically follows a Binomial distribution with parameters N (the population size) and 0.037 (the expected female frequency in the pool of seeds from which generation 2 was sampled, see appendix). The observed distributions of female number at generation 2 were globally consistent with these predictions (Figure 2). However, there was a significant excess of small frequencies at large population size (Chi-square goodness-of-fit test, $\chi^2 = 15.4$, df = 2, $P < 0.001$), which is probably due to a technical underestimation of female frequency at this generation.

The change in mean female frequency at the metapopulation level between generation 2 and 10 can be related to changes in the distribution of female frequency among populations. In metapopulations with large population size, the observed increase in female frequency (Figure 1) resulted from an increased number of populations with high female frequency (Figure 2); on the contrary, in metapopulations with small population size the decrease in the mean female frequency was due to loss of females in many populations (Figure 2).

Table 2. Testing seed size effect – ANOVA on the total number of fruits

N = 211	DF	Mean square	F value	$P > F$
Seed size	1	22458	21.36	**0.0002**
Progeny	17	1010	2.77	**0.0004**
Progeny × Seed size	17	1055	2.90	**0.0002**
Error	175	364		

Selective advantages for females' progeny: The ANOVA on fruit number revealed a significant effect of seed size on the fruit production of the resulting plants (Table 2). The mean (± SE) fruit production was 53 ± 2 fruits for plants grown from large seeds, and 32 ± 2 fruits for plants grown from small seeds, resulting in a 1.7 selective advantage for larger seeds. Both fruit production and seed-size effect strongly depended on the progeny (Table 2), which might also be an environmental effect: the experimental set-up could not distinguish between progeny and environ-

Table 3. Testing heterosis – ANOVA on the total number of fruits

N = 987	DF	Mean square	*F* value	*P* > *F*
Cross type	3	8915	2.82	0.11
Family	1	16558	8.57	**0.02**
$Mother_{(Fam)}$	7	1932	1.30	0.30
$Father_{(cross \times Fam)}$	8	3156	1.75	0.13
Cross × Family	3	2555	0.91	0.49
Cross × $Mother_{(Fam)}$	21	1549	0.87	0.63
(Mother × $Father)_{(cross \times Fam)}$	28	1816	4.50	<**0.0001**
Error	915	404		

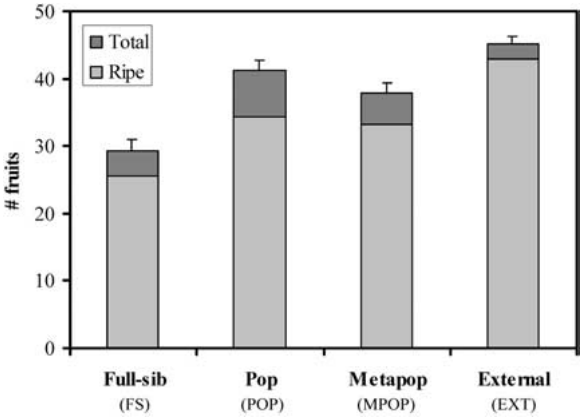

Figure 3. Mean fruit production of individuals originating from a cross between a female plant and fathers that are: full sibs (FS), plants from the same population (POP), plants from the same metapopulation (MPOP) or unrelated plants (EXT). Errors bars represent standard errors, calculated over all individuals within crosses.

mental effects, because seeds from a same progeny were all grown in a single pot.

There was a tendency for a heterosis effect (Figure 3), with greater fruit production for less inbred individuals. This effect was not significant in an ANOVA on the total number of fruits (P = 0.11, Table 3), but there was a significant effect of cross type when the ANOVA was conducted on number of ripe fruits, a variable that combines precocity and total fruit set (Figure 3, P = 0.03). When pairwise comparisons were performed, there was a significant difference in total fruit production only when comparing crosses between full-sibs (FS) and crosses between unrelated individuals (EXT) (P = 0.02). There was no *Mother* or *Father* effect, probably due to the fact that mothers within a family and fathers within a cross type were genetically very close to each other, but there

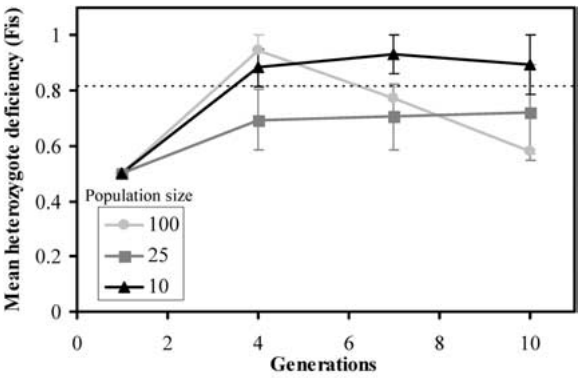

Figure 4. Change in mean heterozygote deficiency (F_{is}) in the three population size treatments. Each dot represents the mean F_{is} in the two metapopulations with same population size and error bars represent standard errors, calculated on the two metapopulation values. The F_{is} was not estimated at generation 1; the theoretical value in the F_2 (0.45) is plotted. The dotted line represents the expected equilibrium F_{is} in an isolated population under 10% outcrossing.

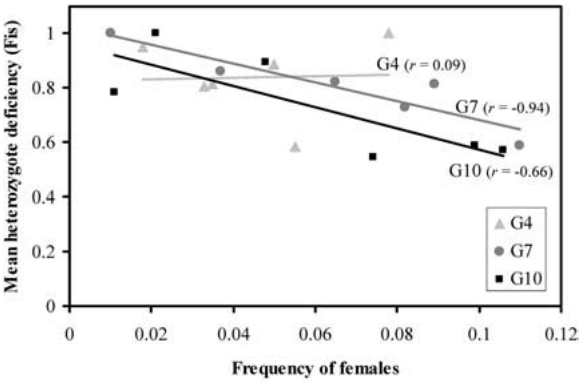

Figure 5. Mean heterozygote deficiency (F_{is}) as a function of female frequency, at generation 4 (triangle), generation 7 (circles) and generation 10 (squares). Each dot represents one metapopulation. The equations of the regressions are the following: $y = 0.27x + 0.83$ (G4), $y = -3.47x + 1.03$ (G7) and $y = -3.89x + 0.96$ (G10).

was a strong interaction between *Mother* and *Father* (Table 3).

Consequences for the heterozygote deficiency: As expected from the highly selfing mating system, the F_{is} increased until generation 4, regardless of population size, as already shown in a previous paper (Figure 4 and Lavigne et al. 2001). Further change in F_{is} depended on population size: high and intermediate F_{is} were maintained at small and medium population size respectively, and F_{is} decreased to a mean value of 0.58 (± 0.01) in metapopulations with large population sizes. The latter value was signifi-

cantly different from the expected F_{is} value under 90% selfing (expected F_{is} in an isolated population = s / (2 – s) = 0.82, t = –30, df = 1, P = 0.02). The behaviour of F_{is} was correlated with the frequency of females in the metapopulations (Figure 5): at generation 7 there was a negative correlation between the observed F_{is} and female frequency (Spearman correlation coefficient, r = –0.94, n = 6, P = 0.005). This relationship also existed, to a lesser extent, at generation 10 (Spearman correlation coefficient, r = –0.66, n = 6, P = 0.16), but was not observed at generation 4 (Spearman correlation coefficient, r = 0.09, n = 6, P = 0.87).

Discussion

Our results confirm that introduction of a nuclear male-sterility gene and correct artificial management of females can modify the outcrossing rate in managed plant populations: outcrossing was increased to 10% in some populations. At the population level, the achievability of this artificial increase did not depend directly on female frequency, but was subordinated to the existence of females, which in turn was under the control of drift and selection for females.

Effect of drift vs. selection on female maintenance and heterozygote deficiency: Females were maintained in most populations at large population sizes (25 or 100 plants per population) over at least 10 generations, but were lost when population size was too small (10 plants per population). The population size effect on female maintenance could be attributed to sampling effects: At small population size (N = 10), the probability P of sampling a population with no female is high ($P = (1-p)^{10}$, where p is the frequency of females in the pool of seeds, $P > 0.1$ for all $p < 0.2$). In addition, females in these populations cannot benefit from the positive frequency-dependent selection that was imposed by the experimental set-up. The selective advantage for females versus hermaphrodites (equation 1) is a decreasing function of female frequency and is larger than 1 only when female frequency is smaller than 0.1. In a small population, the frequency of females is either 0 (no female) or larger than 0.1 (at least one plant in 10; no selective advantage for one female, counterselection for females at higher frequencies). Female frequency therefore rapidly fell to zero in most such populations (Figure 2), and females did not reappear via selfing of heterozygous plants or migration. Under larger population sizes, the probability that females disappear from one population decreases due to frequency-dependent selection and limited sampling effects, and there were fewer populations without females (Figure 2). In metapopulations with 100 plants per population, virtually all populations contained at least one female during the 10 generations. On the contrary, several populations (around 15 of 40) lacked females in metapopulations with 25 plants per populations, but, thanks to migration and selfing of heterozygous plants, females were reintroduced and populations lacking females were not the same from one generation to another.

As expected, the level of heterozygote deficiency after 10 generations of dynamic management depended, at least partly, on the outcrossing rate. When population size was large (N = 100 plants), the outcrossing rate was maintained around 10% because females were always present in most populations. As a result, the heterozygote deficiency at suspected equilibrium was smaller than usually observed in natural populations of predominantly selfing *A. thaliana* (mean ($\pm$ SE) F_{is} = 0.58 $\pm$ 0.01 at generation 10 in the experimental metapopulations, F_{is} = 0.994 $\pm$ 0.002, e.g., in natural British populations (Abbott and Gomes 1989)). On the other hand, in metapopulations with smaller population sizes (25 and 10 plants per population), the mean F_{is} were 0.72 $\pm$ 0.17 and 0.89 $\pm$ 0.11 respectively at generation 10, which is partly due to a moderate to severe decrease in effective outcrossing rates because of the loss of females in many populations.

Selection on the progeny of females: In metapopulations with 100 plants per population, the F_{is} values were smaller than expected under 10% outcrossing (Figure 4). This additional decrease in the level of heterozygote deficiency could be attributable to positive selection on offspring of females. Because they are produced via obligate cross-pollination, females' offspring are more heterozygous in average than hermaphrodites' offspring, their selection therefore results in a decrease in the population mean F_{is}. We found evidence of two possible mechanisms for the selection of females' offspring: a seed-size effect (Table 2) and a heterosis effect (Figure 3, Table 3). These mechanisms conferred a selective advantage around 1.7 and 1.3 respectively for offspring of females vs. hermaphrodites. The selective advantage due to heterosis was estimated as the ratio of the mean seed production of crosses between plants from

the same population to the mean seed production of crosses between full-sibs (Figure 3). These estimates are however possibly biased: the selective advantage conferred by large seeds was probably overestimated, because the mean difference between small and large seeds in the test experiment was larger than observed between progenies from females and hermaphrodites. On the contrary, heterosis might have been underestimated, because female plants were used to perform the crosses, and we compared only full-sibs crosses, not selfing, to other crosses. The existence of heterosis was not clearly demonstrated by the cross experiment: a significant difference between fruit production of plants coming from a cross between full-sibs and from a cross between unrelated plants was found (FS and EXT, Figure 3), but there was no significant difference between full-sib crosses and crosses between plants from the same population or metapopulation. Only this latter comparison (FS vs. POP or, to a lesser extent, MPOP) is relevant in the experimental metapopulations. This absence of significant difference between FS and (POP or MPOP) is either due to an underestimation of heterosis or to an absence of heterosis in this particular experiment; the possible heterosis effects should therefore be considered with caution.

The selection for females' progeny also resulted in an increase in female frequency in large populations: the allele conferring male-sterility was indirectly selected in the progeny of females, which was composed of more than half hermaphroditic plants (MSms) producing $\frac{1}{4}$ females in the next generation. The increase in mean female frequency depended on population size, because of sampling effects that masked the effects of selection: in metapopulations with a large population size (N = 100), females were always present and selection was efficient in all populations, resulting in a relatively small F_{is} and in a clear increase in female frequency. Under intermediate population sizes (N = 25), there was an apparent equilibrium between the loss of females by sampling and their restoration by migration or selfing of heterozygotes, which led to a higher mean F_{is} and to a mean female frequency around the expectation (5%). However, when the populations containing no females were removed from calculations, the mean female frequency in these metapopulations at generation 10 was 0.10 ± 0.02, revealing the efficiency of selection in the presence of females. Finally, the evolution of metapopulations with a small population size seemed to be mainly driven by sampling effects (high F_{is}, extinction of females).

Because of these selection and drift effects, which influence both female frequency and heterozygote deficiency, a significant correlation was found between female frequency and F_{is} (Figure 5). This correlation is usually expected in natural populations containing both females and hermaphrodites (Gouyon and Couvet 1987), but for different reasons: in naturally polymorphic populations, the relationship between female frequency and level of genetic diversity is causal, i.e., more females imply a larger outcrossing rate, and thus a lower level of inbreeding. In this specific situation, the correlation was partly due to a causal relationship between female frequency and outcrossing rates (the latter falling to zero when females disappeared from one population), but also to selection on offspring of females, increasing the frequency of the male-sterility allele and decreasing the level of heterozygote deficiency.

Applicability of an artificial increase in outcrossing rate to the dynamic management of crop species and wild species: Our results are likely to be applicable to real cases of conservation of crop species and even wild species, although we conducted the study using *Arabidopsis thaliana*, a model species whose genetics is well known. First, nuclear male-sterile lines are available for most selfing crop species and the methodology we propose can be applied as is in a dynamic management scheme. Second, several self-incompatibility loci and nuclear or cytoplasmic determinisms of sex are also known in wild species (e.g., Haring et al. 1990; Lewis 1942). The manipulation of outcrossing rates is therefore conceivable and is all the more important that dynamic management plays a central role in the conservation of wild species, whose seeds are not always compatible with long-term storage. Male-sterility might in particular be a relevant tool for the conservation of wild species. For example, this experiment could be used as a model to manipulate the outcrossing rate in gynodioecious species (7% of all species) although, of course, the genetic determinism of male-sterility would be different. Moreover, mutations conferring recessive genic male-sterility seem to arise frequently (Horner and Palmer 1995) even in natural populations (Lewis 1942) and such mutants may be available for rather unstudied wild species.

Conclusions: The introduction of a male-sterility allele proved useful in terms of conservation of genetic resources, at least in the cases where females were

maintained through time: the level of heterozygote deficiency for neutral markers was decreased within populations via a modification of outcrossing rates. This increase in outcrossing rates and decrease in F_{is} are expected to result in the maintenance of higher levels of neutral genetic variation both at the population and metapopulation levels, via larger effective population sizes (Wang and Caballero 1999) and larger effective recombination rates. Moreover, genetic diversity might also be enhanced due to the increase in female frequency resulting from the selective advantage for offspring of females: the more females participate in outcrosses, the higher the level of genetic variation in their total progeny. These possible effects of outcrossing and selection of heterozygotes on the level of genetic variation were however not directly testable in this experiment, because of the confounding effects of drift. Outcrossing and selection increased with population size, and a larger amount of genetic variation in large populations can either be explained by higher outcrossing rates, stronger selection on heterozygotes, weaker effects of drift, or a combination of these mechanisms. Yet, two more points have to be considered to confirm these encouraging effects of increased outcrossing on the maintenance of neutral genetic diversity. First, because conservation genetics aims at preserving the evolutionary potential of managed populations, it remains to be tested whether an increase in outcrossing rate not only has a measurable impact on neutral diversity but also on selected quantitative genetic variation. Second, female plants were maintained here via a combination of an artificial selective advantage for females plus an unanticipated selective advantage for offspring of females. Thanks to this combination, it was possible to maintain an at least 10% outcrossing rate, but the maintenance of females without the unexpected advantage for their progeny would have been more difficult, especially at intermediate population size, where the frequency of females was only maintained at 5% despite this selective advantage. This indicates that maintaining female individuals in populations or metapopulations of a species displaying no selective advantage for females can only be considered for rather large population sizes.

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Appendix

Expected change in female frequency

In the case of a nuclear recessive allele conferring male-sterility, the change in female frequency can be modelled as follows. Let M and m be the alleles conferring male-fertility and male-sterility respectively and q_t the frequency of m allele among hermaphrodites (and therefore in the pollen pool) at generation t ($q_t = \frac{1}{2} f(Mm)_t / [f(MM)_t + f(Mm)_t]$). The frequency of genotypes MM and Mm among hermaphrodites are thus $(1 - 2q)$ and $2q$ respectively. Under the hypotheses that (1) hermaphrodites are complete selfers and (2) allelic and genotypic frequencies are identical in all populations, the genotypic frequencies at generation t can be written as follows:

$$f(mm)_t = 0.1(1 + 0.02)q_{t-1} + (0.88 + 0.9 \times 0.02)(2q_{t-1}/4)$$
$$f(Mm)_t = 0.1(1 + 0.02)(1-q_{t-1}) + (0.88 + 0.9 \times 0.02)(2q_{t-1}/2)$$
$$f(MM)_t = (0.88 + 0.9 \times 0.02)[(2q_{t-1}/4) + (1 - 2q_{t-1})]$$

Note that these recurrence equations for genotypic frequencies do not depend on the genotypic frequencies in the preceding generation, as specified in the material and methods section. Given the initial crosses (Table 1), the expected initial frequencies of females and heterozygous hermaphrodites are 0.0625 and 0.125 respectively. Using these values and the recurrence equations, the evolution of female frequency is plotted on Figure A1.

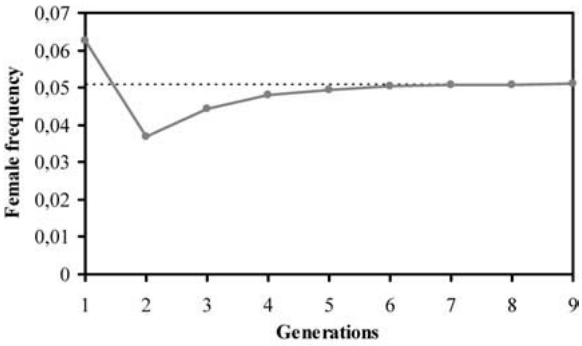

Figure A1. Expected change in female frequency in the absence of selective advantage for the progeny of females.

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