

## ORIGINAL ARTICLE

Population size, self-incompatibility and genetic rescue in diploid and tetraploid races of *Rutidosia leptorrhynchoides* (Asteraceae)M Pickup<sup>1,2</sup> and AG Young<sup>1</sup><sup>1</sup>CSIRO Plant Industry, Canberra, Australian Capital Territory, Australia and <sup>2</sup>Department of Botany and Zoology, Australian National University, Canberra, Australian Capital Territory, Australia

Self-incompatibility systems function to prevent inbreeding, and work effectively in large, genetically diverse populations. However, a decrease in population size can reduce genetic diversity at the self-incompatibility locus, which leads to a reduction in mate availability and has important demographic implications for small populations. Currently, little is known about the response of self-incompatible polyploid species to a reduction in population size. In *Rutidosia leptorrhynchoides* there was a significant decrease in the within-population probability of fertilization with a decline in population size for diploid populations and a marginally significant relationship for tetraploid populations, suggesting that in small populations of both chromosome races fertilization success is reduced due to a decrease in self-incompatibility allele (S-allele) diversity. There was no significant difference between the slopes of the fertility–population size relationship for diploid and tetraploid populations which indicates a similar

rate of decline in fertilization success with population size for both chromosome races. Fertilization success increased when crosses were undertaken between populations and this was significantly related to population size for diploid and tetraploid populations, indicating that small populations gain the greatest benefit to fertilization success from crossing between populations. For tetraploid populations the benefits of crossing between populations tended to decline more rapidly with increasing population size. These results suggest that for small populations that have reduced fertilization success, genetic rescue by introducing new genetic material from other populations is an important means of ameliorating mate limitation issues associated with reduced S-allele diversity in both diploid and tetraploid races.

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## Introduction

Plant self-incompatibility (SI) systems function to avoid the deleterious effects of inbreeding by preventing self-pollination or pollination among related individuals that share self-incompatibility alleles (S-alleles) (De Nettancourt, 1977; Hiscock and Tabah, 2003; Mable *et al.*, 2003). SI systems (both sporophytic and gametophytic; for review see Castric and Vekemans, 2004) are advantageous in large, genetically diverse populations where negative frequency-dependent selection functions to maintain high S-allele diversity (Wright, 1939). However, a reduction in population size in self-incompatible species can lead to a reduction in genetic diversity at the self-incompatibility locus (S-locus), and can have important demographic consequences for small populations due to a reduced availability of compatible mates (Byers and Meagher, 1992; Young *et al.*, 2000b). The adaptive response of self-incompatible species to mate

limitation associated with a decline in population size will depend on the type of SI, but may range from the breakdown of SI to complete maintenance of SI, mostly in response to strong inbreeding depression. Therefore, any shift in mating system in response to population declines will depend on both the degree of flexibility in the SI system (De Nettancourt, 1977; Levin, 1996) and the strength of inbreeding depression (Levin, 1996).

For some self-incompatible species, population declines or severe founder effects have resulted in dissolution of the SI system and selection for self-compatibility to increase reproductive assurance, for example *Aster furcatus* (Reinartz and Les, 1994) and *Scalesia affinis* (Nielsen *et al.*, 2003). Fluctuations in SI in response to dramatic declines in population size associated with colonization dynamics have also been observed in *Crepis sancta* (Cheptou *et al.*, 2002). For species where there is inherent flexibility within the SI system (pseudo-self-compatibility) (De Nettancourt, 1977; Levin, 1996), mate availability in populations with reduced S-allele diversity can be maintained by the action of modifier loci unlinked to the S-locus (Hiscock, 2000; Good-Avila and Stephenson, 2002) or by the action of a cryptic gametophytic SI system (Lewis *et al.*, 1988; Hiscock, 2000). However, the strength of inbreeding depression is an important factor that determines the maintenance of SI

Correspondence: M Pickup, CSIRO Plant Industry, Department of Botany and Zoology, Australian National University, Clunies Ross Drive, Canberra, Australian Capital Territory, 2601, Australia.

E-mail: melinda.pickup@csiro.au

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(Levin, 1996; Brennan *et al.*, 2005), such that there is a balance between maintaining reproductive assurance and avoiding the potential deleterious effects of inbreeding. For example, Brennan *et al.* (2005) conclude that severe inbreeding depression has maintained the strong SI system in *Senecio squalidus* throughout bottlenecks inherent in the colonization process. In this case, increased dominance relationships between S-alleles are thought to have evolved to increase mate availability in small populations with low S-allele numbers (Brennan *et al.*, 2003) as is predicted by theory (Byers and Meagher, 1992; Schierup *et al.*, 1997).

For species that maintain strong SI, the loss of S-allele diversity can have both genetic and demographic effects in small populations. Genetic effects include increased biparental inbreeding and an increase in the effects of genetic drift as effective population size declines (Young *et al.*, 2000b). However, for the S-locus, negative frequency-dependent selection may counteract the loss of diversity through genetic drift compared to neutral loci (Schierup *et al.*, 1998). The demographic effects of reduced S-allele diversity in small populations include a reduction in the proportion of compatible mates (mate availability) which in turn can lead to a reduction in mean population seed set and greater interplant variance in paternal fitness, as individuals with unique S-genotypes are selected for (Byers and Meagher, 1992; Young *et al.*, 2000b). Selection for maternal fitness (fecundity selection) due to low mate availability may also act in small populations, especially in sporophytic SI systems where there is codominance between S-alleles and when there is a low number and/or diversity of pollen donors (Vekemans *et al.*, 1998). Even though fecundity selection is thought to counteract the reduction in mate availability in small populations by helping to maintain high allelic diversity at the SI locus (Vekemans *et al.*, 1998; Castric and Vekemans, 2004), the loss of rare S-alleles through genetic drift, especially in systems where there are strong dominance relationships, means that there can be a marked reduction in compatible mates in generations immediately following a decrease in population size. These effects can have important implications for long-term population viability, especially for species where seed production is the primary factor limiting recruitment (either within populations, or between them in a meta-population context), rather than other demographic processes such as the availability of suitable microsites or negative density dependence (Kirchner *et al.*, 2006). For example, in some populations of the endangered Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*) all extant individuals were of the same incompatibility type, which ultimately led to population extinction (DeMauro, 1993). Another example is the endangered daisy *R. leptorrhynchoides*, where small diploid populations showed a significant reduction in the number of S-alleles that translated directly into a decrease in mate availability and fecundity (Young *et al.*, 2000b). Consequently, for species that maintain strong SI, introducing new S-alleles and thereby increasing mate availability and reproductive success (genetic rescue) may be an important conservation measure to ameliorate the deleterious effects of reduced diversity at the S-locus (Tallmon *et al.*, 2004). Considering small populations are more likely to lose S-alleles through population bottlenecks and genetic drift, smaller populations are expected

to experience the greatest increase in fecundity through introducing novel S-alleles from other populations.

Currently, little is known about the response of polyploid populations to a reduction in population size in relation to genetic diversity at the S-locus, although theory predicts that they may respond quite differently to diploid populations. On the one hand, polyploids (both allopolyploid and autopolyploids) are expected to maintain higher genetic diversity (Bever and Felber, 1992), and this has been demonstrated empirically for neutral loci for intraspecific comparisons (Brown and Young, 2000; Mahy *et al.*, 2000; Hardy and Vekemans, 2001) and between autotetraploid and closely related diploid species (Hokanson and Hancock, 1998; Ng *et al.*, 2004). This suggests that polyploid populations may maintain higher levels of diversity for a given population size. If so, small populations of polyploids would maintain higher fertilization success than diploid populations of similar size. On the other hand, since polyploids have a greater number of alleles per individual, there may be a greater likelihood of matching S-alleles, which means that greater mate limitation in small populations of polyploidy species may be expected. These two processes may also interact to alter mate limitation and the response of polyploid populations to a decline in population size.

There are two primary methods of directly estimating S-allele diversity; the first of these involves undertaking extensive diallel crossing experiments (Karron *et al.*, 1990; Kowyama *et al.*, 1994; Young *et al.*, 2000b; Brennan *et al.*, 2006), while the second is by direct examination of molecular markers for plant families where the S-gene complex has been identified (for example in the Brassicaceae (Mable *et al.*, 2003; Glemin *et al.*, 2005; Schierup *et al.*, 2006) and Solanaceae (Richman *et al.*, 1996)). Alternatively, a probability-based approach to estimate the likelihood of fertilization can be used as a surrogate for S-allele diversity and a direct measure of mate availability. This method involves taking a random sample of individuals from within a population and examining the probability of successful fertilization. This study uses a probability-based approach in diploid and tetraploid populations of the endangered grassland daisy *R. leptorrhynchoides* to address the following three questions:

- (1) Does the probability of fertilization within a population relate to population size?
- (2) Does fertilization success increase when crosses are undertaken between populations, and does this relate to population size? And if so,
- (3) Do these relationships differ for diploid and tetraploid populations?

## Methods

### *Rutidosia leptorrhynchoides*

*R. leptorrhynchoides* is an insect-pollinated multistemmed herbaceous perennial with a sporophytic SI system (Young *et al.*, 2000a) as is characteristic of the Asteraceae. This species is endemic to the grasslands and grassy woodlands of south-eastern Australia, which is a highly fragmented vegetation community that has been reduced to approximately 0.5% of its original two million hectare since the mid 1800s (Kirkpatrick *et al.*, 1995; Brown and

Young, 2000). As a result only 20 remnant populations remain (15 diploid and 5 tetraploid), which are distributed in two broad geographical zones; a northern zone ( $<35^{\circ}30'S$ ,  $>148^{\circ}30'E$ ) in New South Wales and the Australian Capital Territory, and a southern zone that extends through central Victoria ( $>37^{\circ}S$ ,  $>145^{\circ}30'E$ ) (Brown and Young, 2000). Populations in the northern zone consist of diploid individuals ( $2n=22$ ), while the southern zone consists of both diploid and autotetraploid ( $2n=44$ ) populations (Brown and Young, 2000; Murray and Young, 2001).

*R. leptorrhynchooides* has a transient seed bank with no long-term storage of seed in the soil and as such relies on seed from the previous year for recruitment (Morgan, 1995a,b). In addition, seed dispersal distances are commonly less than 0.5 m (Morgan, 1995a) which contributes to high spatial genetic structure (Wells and Young, 2002). Previous demographic work by Morgan (1999) showed that small populations have lower and more variable seed set, in line with the predicted demographic outcomes of reduced diversity at the S-locus (Byers and Meagher, 1992). Further work found that small diploid populations have reduced S-allele diversity and a corresponding decrease in mate availability (Young *et al.*, 2000b). Currently, little is known about the levels of inbreeding in populations, although dominance among S-alleles in sporophytic SI systems and high spatial genetic structure (Wells and Young, 2002) may result in biparental inbreeding in this species.

#### Study populations

Our design used pairs of populations, each with a target population for which the effects of crossing were studied and a source population, which was the source of genetic material for between-population crosses. For the diploid populations, 24 population pairs were chosen to span a range of target population sizes (118–95 000 reproductive plants) and geographic distances (0.7–586.2 km) between target and source populations. Reproductive population size was obtained either by direct counts for populations with fewer than 10 000 plants, or for large populations, reproductive population size was estimated according to methods outlined in Young *et al.* (1999). This method involved using 3–6 quadrats ( $10 \times 10$  m or  $30 \times 30$  m) to estimate flowering density, which was then multiplied by population area to estimate population size. Due to the limited number of remaining *R. leptorrhynchooides* populations and the uneven distribution of populations in different geographic distance classes, most populations were used in multiple population pairs. However, population pairs were chosen to minimize the number of times a population was used and to ensure the even distribution of populations as targets and sources. For tetraploid populations, there are only five remaining populations so five population pairs were chosen to best sample the population size–geographic distance spectrum and to ensure that all populations were only used once as a target and once as a source.

#### Pollination experiments and fertilization success

To examine fertilization success within and between populations in each population pair, controlled cross-pollinations were conducted on 12–18 plants each grown from separate open-pollinated seed families that were

randomly chosen from a potential pool of 30–40 mothers collected from each population in summer 2001/02. For populations used in multiple population pairs, where possible (that is, in 19 of 24 diploid population pairs), a different set of open-pollinated seed families were used to undertake the pollination treatments for each pair. This was done to ensure that independence was maintained between pairs that shared either a target or source population, as this is an important assumption of subsequent regression analyses. In each population pair, plants randomly chosen for the pollination experiments were planted into pots containing one-third potting mix, one-third sand and one-third peat moss and grown in glasshouse conditions with temperatures maintained between 15 and 28 °C. To ensure adequate flowering during the winter months, natural light was supplemented with artificial light to ensure a 14-h photoperiod. In each population pair, plants from the target population were randomly paired for the within-population cross-pollinations (WI-POP) and plants from the target population were randomly paired with plants from the source population for the between-population cross-pollinations (BW-POP).

Inflorescences were bagged on opening and remained bagged for the duration of flowering and until the seed had matured and dehisced (4–5 weeks). For the within-population treatment (WI-POP) one inflorescence from each of two randomly chosen plants originating from the target population were gently brushed together to transfer pollen from the inner florets of one plant to the outer florets of the other. For the between-population treatment (BW-POP) one inflorescence from each of two randomly chosen plants originating from the target and source populations were gently brushed together to transfer pollen from the inner florets of one plant to the outer florets of the other. Crosses for each inflorescence were initiated on the day when the first florets in the inflorescence opened and were repeated 3–4 times, every second day, over the following 6–8 days. This was to ensure adequate pollen availability and that the majority of florets in the inflorescence were pollinated, since the hermaphroditic and partially protandrous inflorescences mature from the outermost whorl inwards over a period of 6–8 days. All cross-pollinations were reciprocal (each inflorescence served as a pollen donor and recipient), giving a total of 50–80 crosses per population pair and 1756 crosses for the entire experiment (across the 29 population pairs; 24 diploid and 5 tetraploid).

For each reciprocal cross-pollination, seed was collected from both plants after seed maturation and counted. Cross-pollinations were scored as compatible if the number of seed was greater than six. Although cross-pollinations with up to six seed were classified as incompatible, the majority of incompatible crosses produced 0–2 seed. This seed-set threshold was chosen as it represents the upper 95% confidence interval for seeds produced under self-fertilization. Hence for each reciprocal cross there were three categories of result; +/+ where both sides of the cross were compatible, +/- where one side of the reciprocal cross was compatible, while the other side of the cross was incompatible and -/- where both sides of the reciprocal cross were incompatible.

From this information, the number of +/+, +/- and -/- crosses within populations and between

populations were calculated and the following formula was used to calculate the probability of fertilization  $P(F)$  both within (WI-POP) and between (BW-POP) populations;

$$P(F) = \frac{(x + 2y)}{2T}$$

where  $x$  = number of instances where only one side of the reciprocal cross was successful (+/-);  $y$  = number of instances where both sides of the reciprocal cross were successful (+/+);  $T$  = total number of crosses performed.

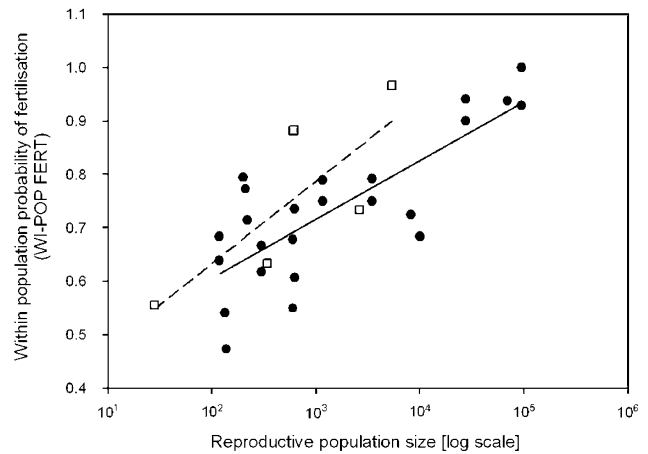
The  $P(F)$  within (WI-POP FERT) and between (BW-POP FERT) populations was then used to calculate the difference in the probability of fertilization (DIFF FERT = BW-POP FERT - WI-POP FERT) that represents the increase in fertilization success by crossing between populations.

### Data analysis

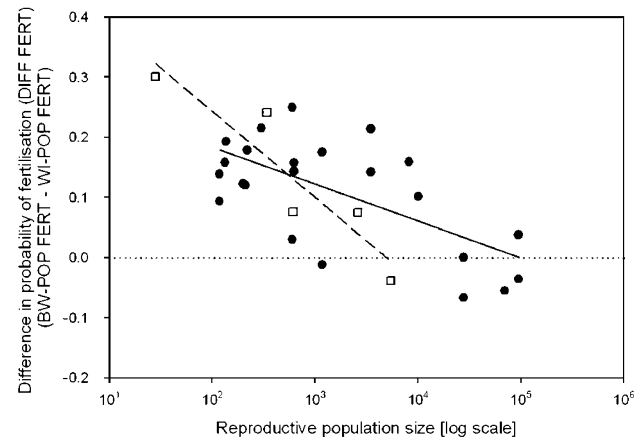
The relationship between population size and; (a) the within-population probability of fertilization (WI-POP FERT), and (b) the difference in the probability of fertilization (DIFF FERT) for diploid and tetraploid population pairs was analyzed using multiple linear regression in Genstat (ninth edition) with target-reproductive population size, source-reproductive population size and geographic distance (all log transformed) included in the model. For both analyses, source-reproductive population size (log) and geographic distance (log) were nonsignificant terms and were therefore removed from the models. These two explanatory variables were included in the initial analysis to examine if the size of the population where the genetic material was sourced and geographic distance between populations were able to explain any of the variation in WI-POP FERT and DIFF FERT. However, in both cases the model that explained the most variation was target-reproductive population size (log). For the full model including ploidy level (diploid and tetraploid) as a grouping factor, analysis of covariance was used to test for homogeneity of slopes for both analyses. Where a common slope could be fitted, differences in elevation (intercept) of slopes were tested by  $t$ -test according to Zar (1999). Assumptions of normality and homogeneity of variances were assessed using residual plots. All statistical tests were significance tested at  $\alpha=0.05$ , however where  $0.05 < P < 0.1$  results were reported as marginally significant.

### Results

There was a significant positive relationship between reproductive population size (log) and within-population probability of fertilization for the diploid populations ( $R^2=0.62$ ;  $P<0.001$ ) and a marginally significant positive relationship for the five tetraploid populations ( $R^2=0.52$ ;  $P=0.10$ ) (Figure 1). For both chromosome races, there were substantial reductions in the probability of successful fertilization across the range of population sizes, with populations approaching 10 000 flowering plants or greater exhibiting unrestricted mate availability, while populations of a few hundred to a few thousand plants had fertilization rates as low as 50–60%. The slope of the regression line was slightly greater for the



**Figure 1** Within-population probability of fertilization as a function of reproductive population size (log scale) for diploid (●) ( $R^2=0.62$ ;  $P<0.001$ ) and tetraploid (□) ( $R^2=0.52$ ;  $P=0.10$ ) populations of *R. leptorrhynchoides*.



**Figure 2** Difference in the probability of fertilization (between-population probability of fertilization – within-population probability of fertilization) as a function of reproductive population size (log scale) for diploid (●) ( $R^2=0.41$ ;  $P<0.001$ ) and tetraploid (□) ( $R^2=0.80$ ;  $P<0.001$ ) populations of *R. leptorrhynchoides*. The dashed horizontal line represents where the difference in the probability of fertilization was 0.

tetraploids compared to the diploid populations ( $\beta=0.154$  and  $\beta=0.109$ , respectively), but there was no statistically significant difference between these two slopes ( $P>0.1$ ), indicating that the probability of fertilization in diploid and tetraploid populations declines at a similar rate as population size decreases. There was also no significant difference in the intercept of the two regression lines for diploid and tetraploid populations ( $P>0.1$ ).

Interpopulation crossing generally resulted in increases in fertilization success of up to 30% over within-population crosses and the effect was negatively related to population size for both diploid and tetraploid populations ( $R^2=0.41$ ;  $P<0.001$  and  $R^2=0.80$ ;  $P<0.001$ , respectively) (Figure 2). The difference in slopes between the two regression lines was substantial, with the slope for the tetraploid population pairs ( $\beta=-0.144$ ) more than double that for the diploid population pairs ( $\beta=-0.061$ ), but this effect was only marginally significant ( $P=0.06$ ). This trend for a difference in slope

indicates that for tetraploid populations the benefits to fertilization success of crossing between populations decline at a faster rate with increasing population size compared to diploid populations.

## Discussion

Genetic diversity at the S-locus has important demographic consequences for small populations (Byers and Meagher, 1992; Young *et al.*, 2000b). A decline in fertilization success with decreasing population size is consistent with the loss of S-alleles in smaller populations, and was clearly evident in diploid and tetraploid populations of *R. leptorrhynchoides*, such that both chromosome races showed a similar rate of decline in mate availability as population size decreased. These results reinforce and extend the generality of the experimental results of Young *et al.* (2000b) based on diallel crossing of five diploid *R. leptorrhynchoides* populations that showed substantial mate limitation in populations fewer than several hundred plants. Genetic rescue in the form of the introduction of new genetic material can have significant benefits for small populations where mate limitation is apparent due to the loss of genetic diversity at that S-locus (Fischer *et al.*, 2003). For *R. leptorrhynchoides*, the increase in the probability of fertilization when crosses were undertaken between populations was substantial (up to 30%) and, as expected, decreased significantly with increasing population size for both diploid and tetraploid populations indicating that the greatest increase in fertilization success was in small populations with low S-allele diversity. For tetraploid populations, however, the benefits to fertilization success of crossing between populations declined at more than twice the rate of diploid populations as population size increased and this result was marginally significant.

The loss of genetic diversity in small populations through bottlenecks and genetic drift can influence genetic diversity at the S-locus even though negative frequency-dependent selection acts to maintain high S-allele diversity. As a result, when population size is small the process of genetic drift may be more important than negative frequency-dependent selection in determining genetic diversity at the S-locus. Nonetheless, negative frequency-dependent selection should reduce the loss of S-alleles when compared to the loss of diversity at neutral loci (Fischer *et al.*, 2003). However, in diploid populations of *R. leptorrhynchoides*, allozyme and S-allele diversity showed a similar rate of decline with decreasing population size (Young *et al.*, 2000b), suggesting that in this case selection has not been sufficient to counteract the loss of S-alleles through bottlenecks and drift. The result for small populations with reduced S-allele diversity is a higher probability of individuals sharing S-alleles which in turn reduces the proportion of compatible mates in the population and leads to reduced fertilization success (Byers and Meagher, 1992; Nielsen *et al.*, 2003), as indicated in the current study. Fecundity selection on maternal fitness may also act in response to reduced mate availability in small populations and contribute to the maintenance of S-allele diversity (Vekemans *et al.*, 1998; Castric and Vekemans, 2004). Random sampling associated with genetic drift will act to remove low frequency, rare alleles, as has been

demonstrated for *R. leptorrhynchoides* where, for allozyme loci, genetic erosion in small populations was primarily due to the loss of low frequency alleles (Young *et al.*, 1999, 2000b). The effect of drift on S-allele diversity, however, will depend on dominance relationships in the SI system (Schierup *et al.*, 1997; Castric and Vekemans, 2004). Frequency-dependent selection in sporophytic SI systems with dominance hierarchies leads to a skewed frequency distribution of alleles where allele frequencies are inversely related to their dominance. Dominance also acts to increase mate availability in small populations (Vekemans *et al.*, 1998), so the action of drift in removing low-frequency dominant alleles may further decrease fertilization success and mate availability in these systems.

Polyploidy is predicted to have a buffering effect on allelic richness (Bever and Felber, 1992; Young and Brown, 2000), which has been demonstrated empirically for neutral loci in a number of species (Soltis and Reiseberg, 1986; Brown and Young, 2000; Mahy *et al.*, 2000; Young *et al.*, 2000b; Hardy and Vekemans, 2001). Consequently, we may expect tetraploid populations to maintain higher S-allele diversity than diploids of comparable size. Results of this study directly challenge the existing paradigm that suggests that small tetraploid populations would have greater resilience to loss of genetic diversity with a decrease in population size. Diploid and tetraploid populations of *R. leptorrhynchoides* experienced a similar decline in fertilization success with decreasing population size, which is indicative of a similar loss of S-alleles in small populations of both chromosome races. In addition, the increase in fertilization success by crossing between populations was greatest in the small tetraploid populations and decreased more rapidly with increasing population size compared to diploid populations, although this difference in slope was only marginally significant.

There are several possible explanations for the apparent difference in response of the tetraploid populations to interpopulation crossing. The first is that on average tetraploid populations could be more genetically differentiated for S-alleles than diploid populations. This would mean that interpopulation crosses would be more likely to involve novel S-alleles from the source population, thus generating a greater genetic rescue effect in small populations. Indeed, allozyme data indicate that on average tetraploid populations are more differentiated for neutral alleles (Brown and Young, 2000) than their diploid counterparts. However, such a pattern may not hold for S-alleles, which are expected to show less differentiation among populations because negative frequency-dependent selection increases the effective migration of S-alleles between populations by favoring novel or rare immigrant alleles (Schierup *et al.*, 2000; Castric and Vekemans, 2004).

A second possibility is that small tetraploid populations are actually more mate-limited than their diploid counterparts due to either (1) increased probability of matching alleles between tetraploid genotypes or, (2) the additional masking of alleles due to dominance in tetraploid genotypes reduces the power of frequency-dependent selection to maintain even frequencies of S-alleles in small populations, resulting in more skewed S-allele distributions and stronger mate limitation. In either case, greater mate limitation in small tetraploid

populations would explain the greater apparent rescue effect on fertilization success when novel alleles are introduced by crossing between populations. The absence of a statistical difference in the effect of population size on within population fertilization success between diploids and tetraploids (Figure 1), however, suggests that small tetraploid populations are not more mate-limited than their diploid counterparts. Though, given the small sample size on which the tetraploid analysis is based, and the trend for a more rapid decline in mate availability with decreasing population size for tetraploids, this possibility cannot be fully discounted. A previous study involving two diploid and three tetraploid populations of *R. leptorrhynchoides* found that for a given level of relatedness, tetraploids showed a 20–25% decrease in compatibility compared to diploid populations (Young *et al.*, 2000a). Taken together, this suggests that despite the potential for tetraploids to maintain higher levels of genetic diversity, the increased probability of matching S-alleles or greater masking of alleles through dominance may counteract this and lead to greater mate limitation in small populations of tetraploids compared to diploids.

Interpopulation gene flow may play an important role in counteracting the decrease in mate availability in small populations due to reduced genetic diversity at the S-locus (Tallmon *et al.*, 2004; Willi and Fischer, 2005). The predicted demographic outcomes for small populations with reduced S-allele diversity include lower and more variable seed set (Byers and Meagher, 1992), which are trends that have been observed in small populations of a number of self-incompatible species (Widen, 1993; Luijten *et al.*, 2000; Fischer *et al.*, 2003), including *R. leptorrhynchoides* (Morgan, 1999). Recent studies to examine the relationship between population size and fertilization success in species with SI systems found reduced cross-compatibility in small populations (Fischer *et al.*, 2003; Willi *et al.*, 2005). For *Ranunculus reptans*, small populations had a significantly higher number of incompatible crosses, suggesting reduced S-allele diversity in small populations (Willi *et al.*, 2005). For this species, interpopulation crosses significantly increased cross-compatibility which was reflected in higher seed set for between-population compared to within-population crosses (Willi and Fischer, 2005). These results support the findings of this study that small populations experience the greatest increase in fertilization success when crosses are undertaken between populations.

For many SI species where the number and size of populations have declined, genetic rescue through the introduction of novel S-alleles may be a viable management option to increase long-term population viability through substantial recovery of seed set. For populations of *R. leptorrhynchoides*, demographic modeling suggests a general positive relationship between genetic diversity, mate availability and persistence time (Young *et al.*, 2000b). Furthermore, the introduction of new S-alleles should only have to be undertaken once, and use only a small number of crosses, as selection will act rapidly to establish them in the population within a few generations. The benefits of introducing novel S-alleles into small populations, however, should be examined in the context of a trade-off with any potential negative effects of outbreeding depression. The risk of outbreeding

depression needs to be considered before moving genetic material between populations, although theory predicts that the risk of outbreeding depression would be reduced for self-incompatible compared to self-compatible species, due to lower population differentiation in self-incompatible species (Dudash and Fenster, 2000). Moreover, interpopulation crosses may also result in heterosis, particularly for small populations suffering from inbreeding (Dudash and Fenster, 2000; Willi and Fischer, 2005). Therefore, rather than a trade-off, introducing new genetic material into small populations may result in a twofold benefit to population viability; first from the benefits to fecundity through the restoration of S-allele diversity, and second any fitness gains accrued from heterosis.

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