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Source population characteristics affect heterosis following genetic rescue of fragmented plant populations

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Understanding the relative importance of heterosis and outbreeding depression over multiple generations is a key question in evolutionary biology and is essential for identifying appropriate genetic sources for population and ecosystem restoration. Here we use 2455 experimental crosses between 12 population pairs of the rare perennial plant *Rutidosia leptorhynchoidea* (Asteraceae) to investigate the multi-generational (F_1 , F_2 , F_3) fitness outcomes of inter-population hybridization. We detected no evidence of outbreeding depression, with inter-population hybrids and backcrosses showing either similar fitness or significant heterosis for fitness components across the three generations. Variation in heterosis among population pairs was best explained by characteristics of the foreign source or home population, and was greatest when the source population was large, with high genetic diversity and low inbreeding, and the home population was small and inbred. Our results indicate that the primary consideration for maximizing progeny fitness following population augmentation or restoration is the use of seed from large, genetically diverse populations.

1. Introduction

Habitat loss and fragmentation have led to the increasing need for population augmentation and habitat restoration to conserve biodiversity. Determining the most appropriate sources of genetic material for genetic rescue [1,2] is critical to ensure the long-term viability of restored or augmented populations [3–5]. Owing to concerns about the loss of local adaptation and reduced progeny fitness through outbreeding depression [6–8], recommendations for sourcing genetic material for genetic rescue and restoration often advocate the use of local genotypes, genetically similar individuals or those from environments that match the transplant site [4,8–11]. Although these recommendations provide an important basis undertaking genetic rescue, there is currently little known about the relative contribution of genetic quality to restoration success. For example, non-local genotypes from large populations with high genetic diversity may provide more appropriate genetic sources for restoration than local genotypes from small, genetically depauperate populations. The potential benefits of genetic rescue may also be greater for small, inbred populations with lower genetic diversity [12,13]. Accordingly, introducing new genotypes may result in a trade-off between the benefits of counteracting inbreeding depression via heterosis, and the loss of fitness through outbreeding depression [8,11]. The dynamics of this trade-off may also change over generations, with greatest heterosis, as a consequence of maximum heterozygosity, expected in the first generation (F_1). In comparison, for outbreeding depression, the dilution of locally adapted genomes may result in reduced fitness of F_1 progeny, but any loss of fitness due to the break-up of favourable epistatic interactions (co-adapted gene complexes) will not occur until the second (F_2) and later generations [14]. Consequently, understanding the relative importance of heterosis versus outbreeding depression over multiple generations are fundamental questions that need to be addressed empirically in conservation.

Inbreeding depression and heterosis have been studied extensively since Darwin ([15], for reviews see [16,17]), whereas the importance of outbreeding depression for the fitness of hybrid progeny is poorly understood [11]. Most studies of outbreeding depression have assessed the fitness consequences of inter-population hybridization for F_1 progeny and only a few have examined these issues over multiple generations [18–23]. Where outbreeding depression has been observed, it has been associated with large spatial scales [24], high genetic divergence [21] or hybridization of distinct sub-species or ecotypes [5]. Understanding correlates of heterosis and outbreeding depression can therefore provide an insight into the mechanisms underlying these processes and thus can help establish useful guidelines when considering genetic rescue.

Spatial scale, environmental variables and population characteristics may predict hybrid fitness, but their explanatory power depends on the underlying mechanisms and relative importance of hybrid vigour (heterosis) and/or hybrid breakdown (outbreeding depression). Geographical distance may correlate with outbreeding depression if genetic divergence and ecological differences among sites (environmental distance) increase with spatial scale [25]. By contrast, in heterogeneous environments with mosaic patterns of environmental variation, environmental distance or genetic divergence based on quantitative traits (Q_{ST}) may more accurately predict the outcomes of inter-population hybridization than geographical distance [23,25]. Even in ecologically similar environments, populations may reach different adaptive peaks or accumulate different coadapted gene complexes [26,27], leading to hybrid breakdown in later generations following inter-population crossing. Thus, genetic differentiation may not follow an isolation-by-distance model nor correlate with environmental variation. Here, molecular genetic distance (F_{ST}) may more effectively predict hybrid fitness than geographical or environmental distance measures if it reflects differentiation in coadapted gene complexes. Characteristics of the local and foreign source population may also contribute to the trade-off between heterosis and outbreeding depression. Elevated inbreeding and the loss of genetic diversity in small populations can result in lower population fitness [28] and greater heterosis when augmenting small compared with large populations [12]. While sourcing from large populations may introduce greater genetic diversity, to our knowledge no studies have examined the importance of population size, genetic diversity or level of inbreeding in the foreign source population for the outcomes of inter-population hybridization.

Here, we examine the multi-generational implications of inter-population hybridization for plant fitness in 12 population pairs of the rare perennial plant *Rutidosis leptorrhynchoides*. We use controlled crosses within and between the home and foreign populations for the 12 pairs to produce control (within home population), F_1 , F_2 , F_3 and backcrossed offspring and compare their fitness in an environment representative of the home population. Specifically, we ask: (i) do inter-population hybrids show heterosis or outbreeding depression? (ii) is this consistent over three generations and for backcrosses to the home and foreign populations? and (iii) can geographical distance (0.7 to approx. 600 km), environmental distance, F_{ST} , Q_{ST} and characteristics of the home and foreign populations (reproductive

population size, allelic diversity (A_E) and inbreeding coefficient (F_{IS}) predict heterosis or outbreeding depression? Our results demonstrate the importance of source population characteristics for seed sourcing in genetic rescue and also provide some novel insights into the processes responsible for heterosis in fragmented plant populations.

2. Material and methods

(a) Species and population pairs

Rutidosis leptorrhynchoides F. Muell. (Asteraceae) is a multi-stemmed, herbaceous, insect-pollinated perennial with a sporophytic self-incompatibility system [29] that is endemic to highly fragmented temperate grasslands and grassy woodland communities of southeastern Australia. The 15 remaining diploid populations are distributed in two broad geographical zones; a northern zone in New South Wales and the Australian Capital Territory (less than 35°30' S, greater than 148°30' E), and a southern zone that extends through central Victoria (greater than 37° S, greater than 145°30' E; see fig. 1 in [30]). The majority of *R. leptorrhynchoides* populations are less than 1000 plants and the largest consists of approximately 100 000 reproductive individuals. To assess the relative fitness of inter-population hybrids compared with progeny from crosses between plants from the home population, we chose 12 population pairs representing a range of spatial scales from less than 1 to 600 km (the first population is the home and the second the foreign population (distance between populations, kilometres); LW-QB (0.7), SR-CC (1.5), MA-BA (4.0), HH-MA (8.0), QB-RH (9.6), CR-LW (15.2), RH-CF (34.8), MJ-GB (71.9), GB-PO (78.9), SR-TR (506.2), CF-SA (516.0), GB-SA (575.1); see also the electronic supplementary material, table S1). Owing to the limited number of remaining *R. leptorrhynchoides* populations, site access restrictions and their uneven distribution in different geographical distance classes, some populations were used in multiple pairs. Population pairs were, however, selected to ensure replication at each distance class while minimizing the number of times a population was included (only GB and SR are used twice as a home population). While the use of populations in multiple pairs does raise issues associated with the assumption of independence, each pair is treated as independent and unique maternal families were used in each pair. We also correct the alpha level for the use of populations in multiple pairs (see §§3 and 4).

(b) Seed collection and crossing experiment

To produce the control (within home population progeny) and inter-population hybrids (F_1 , F_2 , F_3 and backcrosses) for the 12 population pairs, we undertook a multi-generational crossing experiment from November 2002 until January 2005 (figure 1a). Parental material for each population pair was obtained by collecting seed for the home and foreign populations from one to three open-pollinated inflorescences of 30–60 maternal plants. Twelve to eighteen maternal plants were randomly chosen for each population in a pair. Four seeds from all families were then germinated on a moist filter paper in a growth cabinet maintained at 20°C with a 12 L : 12 D cycle. We then transplanted a randomly chosen seedling from each family into 20 cm 1 l capacity pot containing a soil mix of equal parts potting mix, sand and peat moss, and these were grown in a glasshouse maintained at 15–28°C. We supplemented with artificial light to ensure a year-round 14 hr photoperiod.

For the 12 population pairs, we produced control (home × home), F_1 (home × foreign), F_2 (F_1 × F_1) and backcrosses to the home ($BC_{F_1 \times H}$; F_1 × home) and foreign ($BC_{F_1 \times F}$; F_1 × foreign) populations. In addition, for five pairs (SR-CC, HH-MA, MJ-CF, GB-PO and SR-TR), F_3 (F_2 × F_2), backcrosses to the home

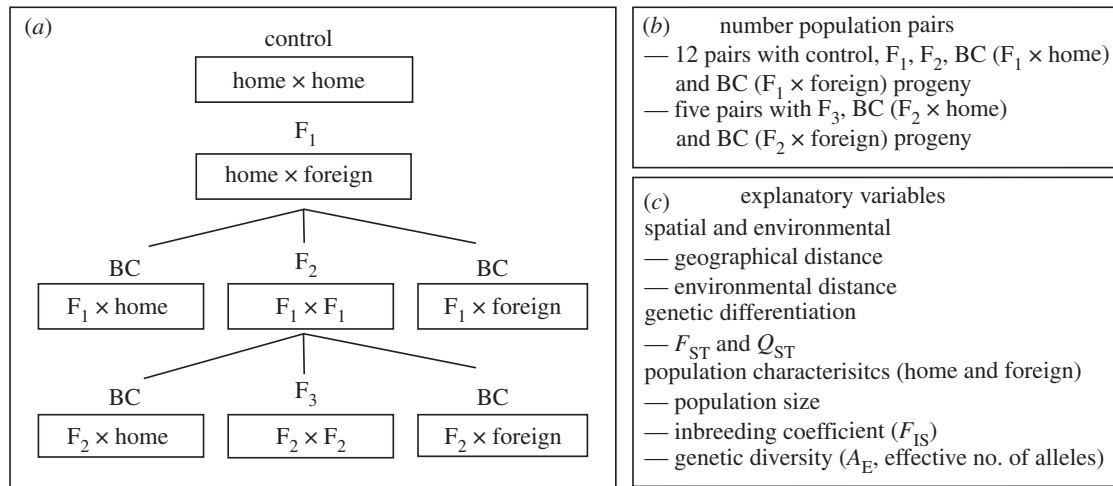


Figure 1. (a) The design of the crossing experiment used to generate the eight progeny types; control (home × home), F₁ (home × foreign), F₂ (F₁ × F₁), backcross (BC) (F₁ × home), backcross (BC) (F₁ × foreign), F₃ (F₂ × F₂), backcross (BC) (F₂ × home) and backcross (BC) (F₂ × foreign). (b) The number of population pairs with each crossing treatment. (c) The predictor variables used to explain the among population pair variation in progeny fitness.

(BC_{F₂ × H}; F₂ × home) and foreign (BC_{F₂ × F}; F₂ × foreign) populations were produced (figure 1a,b, see also the electronic supplementary material, table S2). Each population pair contained 10–15 maternal crossing lines (crosses that originated from a single maternal family). We used a sub-sample of six maternal crossing lines for the five population pairs with F₃ and backcrossed progeny.

We used the following pollination protocol to produce the control and hybrid progeny. We randomly paired plants for all cross types in each population pair. Crosses were initiated on the day the first florets opened and were repeated three to four times, every second day. We gently brushed together a single inflorescence from each of the two randomly chosen plants to transfer pollen. The inflorescences were bagged on opening and between pollinations, and remained bagged until the seed had matured and dehisced (4 to 5 weeks). All cross-pollinations were reciprocal, where each inflorescence served as a pollen donor and recipient. We collected and counted seed for each reciprocal cross-pollination. Some crosses were unsuccessful owing to matching of self-incompatibility alleles (S-alleles) [31]. For these crosses, plants were randomly re-paired and the cross repeated. To produce each subsequent generation (F₂, F₃ and backcrosses), we randomly chose two seed from each side of the reciprocal cross for the 10–15 maternal lines in each population pair and germinated them using the same protocols as the parental generation. We then randomly chose one of these plants at reproductive maturity (approx. 12–16 weeks). The number of reciprocal crosses within each population pair ranged from 150 to 240, so that across all population pairs this experiment involved 2455 cross-pollinations.

(c) Fitness experiment

To examine the consequences of inter-population hybridization for progeny fitness, we compared their fitness with control (within home population) progeny in an environment representative of the home population (see below). From multivariate analysis of bioclimatic and soil (edaphic) variables, we found that populations of *R. leptorrhynchoides* divide into two climatic zones (north and south), but within each climate zone, edaphic variation was the primary driver of environmental heterogeneity [30]. Consequently, our experimental approach involved growing progeny from each population pair in soil collected from the home population and in an outdoor enclosure at CSIRO Plant Industry in Canberra, Australian Capital Territory (35°16'23" S, 149°06'42" E) so that plants experienced daily and seasonal climatic conditions

(variation in temperature and moisture) that were representative of the home site (northern climate zone). The soil (100–3121) was collected from multiple locations within each home population. The overall climate at this location is representative of the local population for all population pairs assessed because all local populations were from the northern climate zone [30]. Consequently, differences in soil were the main driver of environmental differences among the nine population pairs where the home and foreign populations were from the northern climate zone. For the three pairs where the foreign population was from the southern climate zone, both soil and climate differences contributed to environmental differences among sites. Previous studies of this species [30] found a correlation between Q_{ST} and environmental distance based on climate and soils. This suggests that these ecological variables are related to the environmental selective pressures in each population. Consequently, although we were unable to assess the importance of other biotic (e.g. competition, pathogens) or abiotic factors, our experimental framework enabled us to compare progeny performance in an environment that captured the local soil and climatic conditions. The soil samples from each population were mixed and used in an 80:20 ratio with river sand (to improve drainage) in 10 cm diameter 0.5 l capacity pots.

We used 12 maternal lines for each population pair (except CF-SA, $n = 10$). For each maternal line and cross type, we randomly selected one side of the reciprocal cross and counted and bulk weighted 12 seed. Seeds were then cold treated in a refrigerator set at approximately 5°C for 72 h. We planted one to three seeds into each of four pots for each cross type and maternal line in April–May 2005. For each population pair, $n = 200$ –312 pots and for all population pairs $n = 3200$ pots and 9397 planted seed. We grouped together a single pot of each cross type for each maternal line, and each of the four replicated sets were positioned in a complete randomized block design with four blocks. The position of the replicate in each block was arranged using a randomized row and column structure. We supplemented natural precipitation with hand-watering every 1–2 days as required.

(d) Fitness components

To compare the relative performance of inter-population hybrids and progeny from the home population, we measured seedling survival as well as two adult fitness components (at 12 months); number of inflorescences (reproduction) and biomass. We recorded survival weekly for the first 3 months. For

pots where multiple seedlings had germinated, all seedlings except the one closest to the geometric centre of the pot were removed before competition ensued. At 12 months, we harvested and obtained dried weights for above- and below-ground biomass by drying samples at 70°C for 3 days before weighing them on a four decimal place gram balance. The root mass was washed prior to drying. Together, these provided an estimate of total biomass (hereafter biomass). Previous demographic work for *R. leptorrhynchoides* [32] found that seedling and adult survivorship, as well as adult reproductive characteristics had the highest elasticity values and therefore have a high contribution to population growth rate. Survival from seedling to adult was much higher in our experiment (greater than 95%) compared with observations of plants in the field. Thus, in our experiment, we use biomass as a surrogate for adult survival, as plant size has been shown to be associated with survival in natural populations (A. G. Young 2000, unpublished data).

3. Statistical analysis

(a) The effect of crossing treatment on fitness components

To examine the effect of cross type (control, F_1 , F_2 , F_3 and backcrosses) and population pair (and their interaction) on progeny fitness, we used; (i) a generalized linear model (logistic regression) for seedling survival (pooled across families), (ii) generalized linear mixed models for number of inflorescences (normal distribution, log link function) and (iii) REML linear mixed models for biomass. For all analyses, seed weight was fitted as a covariate, but where it was non-significant was removed from the final model. For all mixed models, cross type and population pair (and their interaction) were fitted as the main effect in the fixed model, while maternal line (nested within population pair), row and column position (in each block) and block were fitted in the random model. Differences between cross types were assessed using least significant difference (LSD; at $\alpha = 0.05$).

(b) Linear regression analysis

We analysed the relation between the difference in fitness between control (within home population progeny) and hybrid progeny (F_1 , F_2 , F_3 and backcrosses) and variables: (i) log home reproductive population size (logHPS), (ii) log foreign reproductive population size (logFPS), (iii) effective number of alleles in the foreign population (A_{EF}), (iv) inbreeding coefficient of the home ($F_{IS}H$) and foreign ($F_{IS}F$) populations, (v) log geographical distance (logGD), (iv) environmental distance (EnvD), (v) Q_{ST} , and (vi) F_{ST} (figure 1c), for each fitness component using multiple (and single) linear regressions. Details on the derivation of each of these explanatory variables can be found in [30], while the values for each population pair are listed in the electronic supplementary material, table S1. We used stepwise ANOVA for model selection to identify the single variable, or combinations of variables that best explained the difference in fitness between control and hybrid progeny. We then analysed identified variables as simple or multiple linear regressions. A maximum of two explanatory variables were used in the multiple regression models for the F_1 , F_2 and backcrosses ($n = 12$) and a single variable used for the F_3 and backcrosses ($n = 5$). Regression analysis of biomass for

the F_1 , F_2 and backcrosses to the foreign population ($BC_{F_1 \times F}$) identified MJ-GB as a high leverage data point. Accordingly, we present the results of the analyses with and without this population pair. Significance was tested at $\alpha = 0.05$; however where $0.05 < p < 0.1$ we report results as marginally significant. To account for the non-independence of population pairs that shared a home population (i.e. SR-CC, SR-TR and GB-PO, GB-SA), we use a Bonferroni correction ($\alpha = 0.05/2$) and test significance of these relations at $\alpha = 0.025$. We used GENSTAT 13th edn (VSN International, Oxford, UK) for all analyses.

4. Results

(a) Fitness of inter-population hybrids compared with progeny from the home population

The fitness of progeny from inter-population crosses over multiple generations (F_1 – F_3 and backcrosses) was either no different or increased (heterosis) compared with progeny from the home population for the majority of population pairs and fitness components. Including all population pairs, significant heterosis was observed for seedling survival ($p = 0.002$), whereas inter-population hybrids had similar fitness to progeny from the home population for biomass ($p = 0.058$) and reproduction (number of inflorescences, $p = 0.076$; table 1). There was, however, variation in heterosis among population pairs for all fitness components. Here, we examine this variation among population pairs in relation to a range of distance matrices and characteristics of the home and foreign source populations (see [30]; figure 1c and electronic supplementary material, table S1). We found that the level of inbreeding (F_{IS}), genetic diversity (A_E) and size of the source and home population were the most consistent predictors of heterosis across a range of fitness components. Heterosis was greater when the foreign population was large and genetically diverse with low levels of inbreeding and the home population small or inbred. We now examine these results in more detail for three fitness components identified as high elasticity traits, seedling survival, biomass and reproduction (number of inflorescences).

(b) Seedling survival

We found that the population characteristics and distance matrices only explained the among population pair variance in seedling survival for F_2 progeny. Here, the inbreeding coefficient of the foreign population ($F_{IS}F$) and environmental distance explained 39 per cent of the variance in seedling survival ($p = 0.043$, see the electronic supplementary material, figure S1), with greater heterosis in the F_2 when the foreign source population had low levels of inbreeding (low F_{IS}) and populations came from similar environments (smaller environmental distance).

(c) Biomass

We found that the inbreeding coefficient ($F_{IS}F$) and reproductive size of the foreign source population explained 32–69% of the variance in biomass of the F_1 (figure 2a; $R^2 = 0.32$, $p = 0.071$, without MJ-GB $R^2 = 0.46$, $p = 0.034$), F_2 (figure 2b; $R^2 = 0.36$, $p = 0.053$, without MJ-GB $R^2 = 0.55$, $p = 0.017$) and backcross progeny ($BC_{F_1 \times FOREIGN}$;

Table 1. Statistical analyses and per cent difference between the control (within home population cross) and each crossing treatment (treatment - control, as % of control) for a number of fitness components of *R. leptorrhynchoides* including all 12 population pairs. (Positive (or negative) values represent the per cent increase (or decrease) in the crossing treatment (F_1 , F_2 , F_3 and backcrosses) compared with the control. Positive (or negative) values in bold indicate significant heterosis (or outbreeding depression); LSD at $\alpha = 0.05$. H is the home (local) population and F is the foreign (source) population.)

fitness component	F	p-value	crossing treatment						
			F_1 (H \times F)	BC ($F_1 \times F$)	F_2 ($F_1 \times F_1$)	BC ($F_1 \times H$)	BC ($F_2 \times F$)	F_3 ($F_2 \times F_2$)	BC ($F_2 \times H$)
seedling survival									
cross type	3.26	0.002	1.0	0.3	0.5	-0.2	1.5	2.7	3.9
population pair	4.48	<0.001							
cross type \times population pair	1.24	0.120							
biomass									
cross type	1.95	0.058	2.1	-0.7	4.4	2.1	-1.0	0.3	1.0
population pair	99.60	<0.001							
cross type \times population pair	0.89	0.698							
number inflorescences									
cross type	1.84	0.076	8.4	11.9	11.7	6.1	-5.0	3.0	-0.2
population pair	31.71	<0.001							
cross type \times population pair	2.18	<0.001							

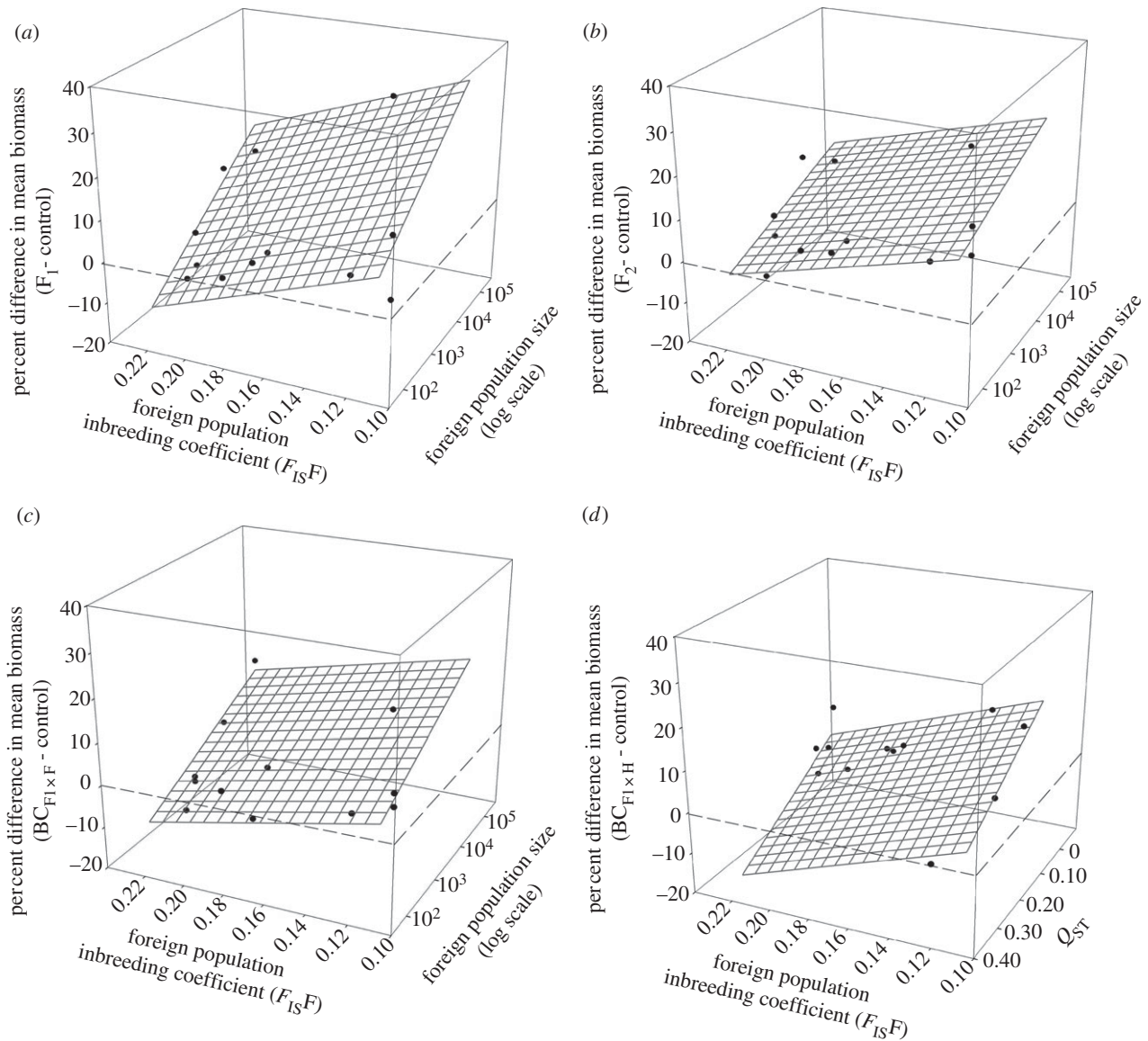


Figure 2. The difference in mean biomass between the control (within home population progeny) and (a) F_1 , (b) F_2 , (c) backcrosses to the foreign population ($BC_{F_1 \times F}$) and (d) backcrosses to the home population ($BC_{F_1 \times H}$), as a function of the inbreeding coefficient of the foreign population ($F_{IS}F$) and foreign population size (FPS) (log scale) for (a), (b) and (c) and quantitative genetic divergence (Q_{ST}) for (d). Values above the dashed horizontal (zero) line represent heterosis and below represent outbreeding depression. The equations for these relations are: (a) $R^2 = 0.32$, $p = 0.071$, biomass = $3.7 - 141.6 \times F_{IS}F + 8.2 \times \text{LogFPS}$, (without MJ-GB: $R^2 = 0.46$, $p = 0.034$), (b) $R^2 = 0.36$, $p = 0.053$, biomass = $14.9 - 130.5 \times F_{IS}F + 4.4 \times \text{LogFPS}$ (without MJ-GB: $R^2 = 0.55$, $p = 0.017$), (c) $R^2 = 0.69$, $p = 0.002$, biomass = $1.1 - 107.4 \times F_{IS}F + 5.9 \times \text{LogFPS}$ (without MJ-GB: $R^2 = 0.59$, $p = 0.012$), (d) $R^2 = 0.60$, $p = 0.006$, biomass = $46.9 - 219.1 \times F_{IS}F - 80.0 \times Q_{ST}$.

figure 2c; $R^2 = 0.69$, $p = 0.002$, without MJ-GB $R^2 = 0.59$, $p = 0.012$), with greater heterosis when the source population was large with low levels of inbreeding. For backcrosses to the home population ($BC_{F_1 \times \text{HOME}}$), the inbreeding coefficient ($F_{IS}F$) of the source population and Q_{ST} explained 60 per cent of the variance in progeny fitness (figure 2d; $R^2 = 0.60$, $p = 0.006$). Here, heterosis was greater in population pairs where the source population had lower levels of inbreeding (low $F_{IS}F$) and where quantitative genetic divergence (Q_{ST}) was low. For the F_3 generation, F_{IS} of the source population ($F_{IS}F$) explained 60 per cent of the variance in progeny fitness ($p = 0.077$; table 2), with greater heterosis when the source population had low levels of inbreeding. By contrast, for backcrosses to the home population ($BC_{F_2 \times \text{HOME}}$), heterosis for biomass was greater when the home population was inbred (table 2; $R^2 = 0.74$, $p = 0.038$).

(d) Reproduction

We found that the genetic diversity of the foreign source population (measured by the effective number of alleles, A_E) was the most consistent predictor of heterosis for the number of inflorescences in the F_1 , F_2 and backcross generations. For the F_1 (figure 3a; $R^2 = 0.36$, $p = 0.023$) and F_2 (figure 3b; $R^2 = 0.43$, $p = 0.012$), the effective number of alleles (A_E) of the source population explained 36–43% of the variance in fitness, with greater heterosis in population pairs where the source population was more diverse. Similarly, the effective number of alleles in the source population explained 33 per cent of the variance in the number of inflorescences for backcross progeny to the foreign population in the second generation ($BC_{F_1 \times \text{FOREIGN}}$, $R^2 = 0.33$, $p = 0.029$; figure 3c). However, for backcrossed progeny to the home population ($BC_{F_1 \times \text{HOME}}$), heterosis was greater when environmental

Table 2. Results of the linear regression analysis examining the effect of the inbreeding coefficient of the home ($F_{IS}H$) and foreign ($F_{IS}F$) populations on the difference in biomass for F_3 and backcrossed progeny compared to the control (within home population cross) for five population pairs of *R. leptorrhynchoides*.

fitness component	cross type	R^2	p -value	explanatory variable	slope	model equation
biomass	$F_3 (F_2 \times F_2)$	0.60	0.077	$F_{IS}F$	–	$y = 16.65 - 99.6 \times F_{IS}F$
	BC ($F_2 \times \text{home}$)	0.74	0.038	$F_{IS}H$	+	$y = -44.4 + 252.9 \times F_{IS}H$

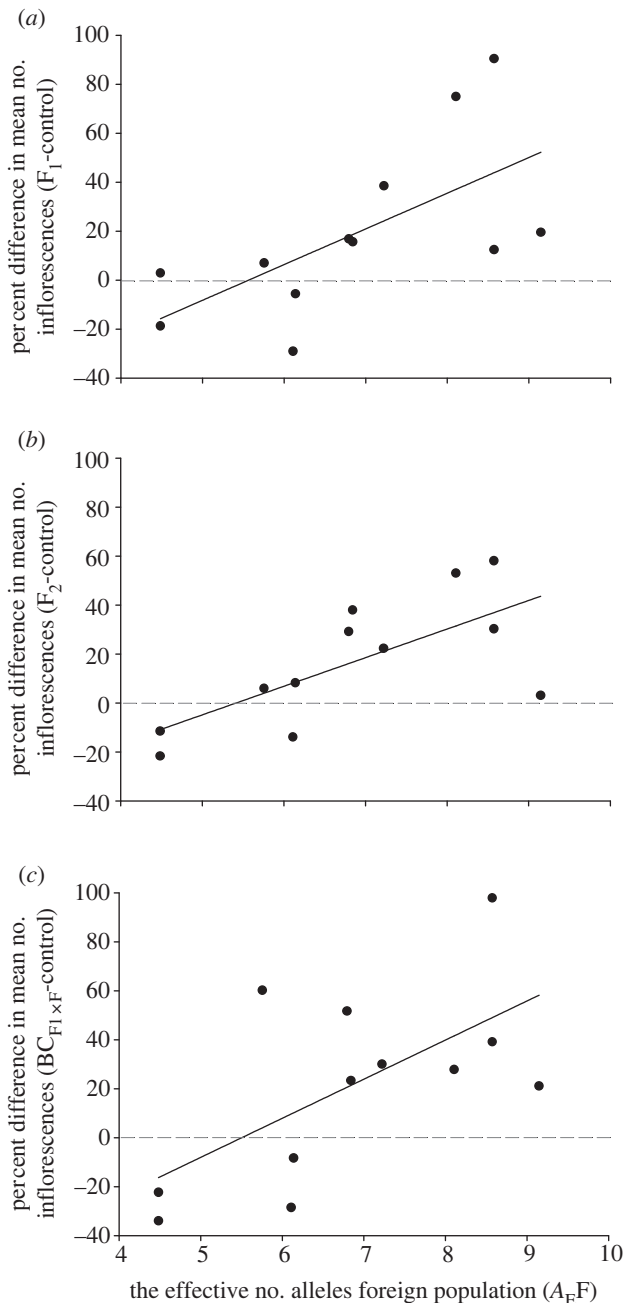


Figure 3. The difference in the mean number of inflorescences (no. inflor.) between the control (within home population progeny) and (a) F_1 , (b) F_2 and (c) backcrosses to the foreign population ($BC_{F_1 \times F}$) as a function of the effective number of alleles in the foreign population (A_{EF}). Values above the dashed horizontal (zero) line represent heterosis and below represent outbreeding depression. The equations for these relations are: (a) $R^2 = 0.36$, $p = 0.023$, no. inflor. = $-81.0 + 14.6 \times A_{EF}$, (b) $R^2 = 0.43$, $p = 0.012$, no. inflor. = $-63.2 + 11.7 \times A_{EF}$ and (c) $R^2 = 0.33$, $p = 0.029$, no. inflor. = $-87.9 + 16.0 \times A_{EF}$.

distance was large and F_{ST} small ($R^2 = 0.58$, $p = 0.008$, electronic supplementary material, figure S2).

5. Discussion

Our study demonstrates the importance of source population characteristics for heterosis following hybridization among fragmented populations of a rare perennial plant. For a range of fitness components and across multiple generations, hybrid progeny fitness was greatest when the foreign source population was large, with high allelic diversity and low levels of inbreeding (low F_{IS}). These results have important implications for understanding potential trade-offs between heterosis and outbreeding depression following genetic rescue. Currently, guidelines regarding the movement of genotypes for population augmentation or ecological restoration advocate the use of local or genetically similar genotypes owing to the risk of outbreeding depression [8]. Our results suggest that for small, inbred populations inter-population crossing results in heterosis, rather than outbreeding depression and that progeny fitness is maximized by sourcing genetic material from large, outbred populations with high genetic diversity. The maintenance of heterosis beyond the first generation also suggests that these effects may be beneficial for population growth rate for several generations. Compared with characteristics of the source population, geographical distance and environmental distance had little power to predict hybrid progeny fitness. Our results highlight the central role of small population processes in the trade-off between heterosis and outbreeding depression following inter-population hybridization.

Genetic drift and inbreeding in small populations result in reduced population fitness owing to loss of genetic diversity and an increase in the expression of mildly deleterious alleles [12,33]. Inter-population hybridization can counter this loss of fitness through either the masking of deleterious recessive alleles or overdominance (heterozygote advantage) [34,35]. For *R. leptorrhynchoides*, inbreeding occurs via mating among close relatives (bi-parental inbreeding) and the loss of mating types (S -alleles) in small populations can further reduce effective population size [36]. As expected from theory [37] and recent studies of inbreeding and outbreeding depression [23,38,39], small, inbred populations of *R. leptorrhynchoides* experienced greater heterosis than large, outbred populations. The novel finding of our study is the importance of these processes in the foreign source population for hybrid progeny fitness. The increase in heterosis when genotypes were sourced from large, genetically diverse populations may result from a number of processes. First,

the increased efficiency of selection in large populations can result in a lower frequency of deleterious recessive alleles, leading to greater heterosis via dominance. While there is less evidence for overdominance [35], this may also contribute to progeny fitness when genotypes are sourced from larger populations with high genetic diversity. Sourcing from populations with higher diversity may provide more variation for the evolution of novel, highly fit genotypes through recombination in the F_2 and subsequent generations [18]. However, this is more likely to effect the variance in fitness rather than the mean. Given the low number of genetic clusters in this species ($K = 3$, see [30] for STRUCTURE analysis), heterosis may be reduced when matings occur between populations belonging to the same genetic cluster. However, we found that heterosis was not limited to pairs where the populations had very different average membership to the three genetic clusters (see the electronic supplementary material, figure S3). This suggests that high genetic diversity or low inbreeding in the source population are the more likely drivers of the observed heterosis, rather than genetic dissimilarity of the home and source populations. Our results are intriguing and although the exact mechanism(s) underlying these patterns are at this stage unclear, they indicate the importance of considering source population characteristics for genetic rescue.

In combination, population size, genetic diversity and the level of inbreeding of the source population consistently explained variation in hybrid performance across a range of fitness components. Population size is often correlated with genetic diversity and fitness [28,40], but not necessarily the level of inbreeding (F_{IS}) [40]. Similarly, we found that population size was related to genetic diversity (A_E ; $R^2 = 0.28$, $p = 0.025$) but not the inbreeding coefficient (F_{IS} ; $p > 0.05$) for *R. leptorrhynchoides* (see the electronic supplementary material, appendix S1). High fine-scale spatial genetic structure in this species [41] may contribute to higher rates of inbreeding in larger populations, so that these two population characteristics explain different components of heterosis. Taken together, these results suggest that population size may not always be an effective surrogate for source population quality and that additional information on inbreeding and the level of genetic diversity can assist in the choice of the most appropriate source populations for restoration.

Geographical distance had very little power to predict hybrid progeny fitness for any of the fitness components or generations. Spatial scale is often used to delineate seed sourcing zones in conservation with emphasis on the use of local genotypes. Along with previous studies where geographical distance had limited value for predicting hybrid progeny fitness [5,24,42], our results suggest that other criteria such as the size and diversity of the source populations are more important considerations for genetic rescue. The importance of environmental distance for progeny fitness in *R. leptorrhynchoides* was generally low, but varied among traits and generations. Consequently, along with characteristics of the source population, ecological differences among sites remain an important consideration when selecting genetic sources for restoration [5,42]. Previous work on adaptive differentiation in *R. leptorrhynchoides* found that local adaptation was generally low, except for some local genotype advantage for biomass when Q_{ST} was high [30]. Here, along with F_{IS} of the source population, we found low Q_{ST}

was associated with greater heterosis for biomass in backcrosses to the home population. This suggests that for traits where local adaptation is related to quantitative genetic divergence, heterosis can be maximized by sourcing from outbred populations that are less phenotypically divergent.

While the importance of genetic divergence for hybrid vigour is well established for crop breeding [43], our results challenge the current paradigm of avoiding the movement of genotypes among divergent natural plant populations [8]. Similar to Willi *et al.* [23], we found that F_{ST} and Q_{ST} had little power to predict either heterosis or outbreeding depression, and that there was no negative effect of gene flow among populations with F_{ST} values of 0.03–0.11 and Q_{ST} values 0.01–0.33. For *R. leptorrhynchoides*, these two matrices were only marginally correlated and ($r = 0.44$, $p = 0.064$), indicating that they reflect different measures of population differentiation. The lack of association between hybrid progeny fitness and F_{ST} over a number of generations suggests that this measure of genetic distance does not reflect patterns of genetic coadaptation. We found that Q_{ST} was not related to any loss of fitness through the dilution of local genes in the F_1 or backcrosses to the foreign population (where progeny had a reduced average proportion of local genes), even though Q_{ST} was associated with ecological differences among populations (Q_{ST} and environmental distance, $r = 0.64$, $p = 0.006$) and local adaptation for some traits [30]. Gene flow among moderately divergent populations of this species is therefore likely to have either no effect or result in hybrid vigour, especially for inbred populations and when the genetic and demographic characteristics of the source populations are considered.

Genetic rescue and the choice of appropriate source populations is a major challenge in conservation and restoration [7,8]. Our results demonstrate that, instead of detrimental effects, mixing genetic material from divergent populations resulted in hybrid vigour when genotypes are sourced from large populations with high genetic diversity and low levels of inbreeding, and that this fitness benefit is maintained over generations. This highlights the importance of genetic and demographic characteristics of both the recipient and source population when considering artificial gene flow among populations. In line with both theory [37,44] and empirical studies [18,23], our study supports the potential role of genetic rescue as a means of increasing the probability of local population persistence. This may be especially important for species where there is a frequency-dependent selection for rare alleles (e.g. *S*-alleles). Consequently, rather than a trade-off between inbreeding and outbreeding, genetic rescue may provide a twofold fitness benefit for self-incompatible species. The first benefit is owing to increased fertilization success [31,39] and, the second is attributable to the multi-generational fitness benefits of heterosis, particularly when immigrants are sourced from large, outbred and genetically diverse populations.

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