Local Adaptation and Outbreeding Depression in Fragmented Populations of *Rutidosis leptorrhynchoides* (Asteraceae)

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By

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DECLARATION

I, Melinda Pickup, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the Department of Botany and Zoology, Australian National University, is wholly my own work unless otherwise referenced or acknowledged. The document had not been submitted for qualification at any other academic institution.

Melinda Pickup

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ABSTRACT

The potential for local adaptation and outbreeding depression are important genetic issues in the restoration of threatened plant species. Transplanting individuals that are ill-adapted to the transplant site will affect the success of restoration attempts, while any reduction in the fitness of offspring of local and foreign plants (inter-population hybrids) can affect the long-term viability of restored populations. Conversely, genetic rescue, though augmenting small populations with new genetic material, may benefit small populations with elevated inbreeding and reduced *S*-allele diversity through both the positive fitness effects of heterosis and by increasing mate availability through the introduction of novel *S*-alleles.

Despite the importance of these issues, there are still only limited data available regarding the magnitude and scale of local adaptation and outbreeding depression in natural plant populations, how they relate to environmental differences between populations and if there is a trade-off with the potential positive effects of introducing new genetic material. *Rutidosis leptorrhynchoides* (Asteraceae) is an endangered perennial herb endemic to the grasslands and grassy woodlands to South-Eastern Australia. Given the widespread distribution and varied size of remnant populations, its high conservation status and sporophytic self-incompatibility system, this species provides an ideal model system to examine local adaptation and outbreeding depression in relation to: (i) population size, (ii) spatial scale, (iii) environmental differences between populations, and (iv) in the context of the potential benefits of genetic rescue for *S*-allele diversity in small populations.

Local adaptation was assessed using a transplant experiment that involved 18 population pairs separated by a range of distances from 0.7 – 600 km. For each population pair, seed from both the Target (local) and Source (foreign) populations were planted into soil from the Target population site and grown in a common climate representative of the Target population. Plants were grown for 24 months and the performance of plants from the Target and Source populations were compared for fitness traits across the life-cycle. The difference in fitness between the local Target and

foreign Source populations was then related to Target and Source population size and geographic and environmental distance between populations.

There was no consistent evidence of local adaptation across a range of fitness traits, with equivalent performance of local and foreign genotypes, local adaptation and foreign genotype advantage all observed in the overall analysis including all population pairs and in a number individual population pairs spanning a range of spatial scales from 0.7 – 600 km. When fitness was assessed across the lifecycle from germination through to reproduction (cumulative fitness), there was, in fact, evidence of an overall foreign genotype advantage, indicating superior performance of the foreign Source population. These results imply that translocation may have both positive and negative outcomes for mean population fitness, depending on the fitness trait measured, and that even when translocating over large spatial scales (up to 600 km), foreign genotypes may have equivalent or greater performance than the local Target population. For above ground biomass and the number of leaves at 12 months, local adaptation was positively related to geographic and environmental distance respectively, suggesting that adaptive differentiation may increase with spatial scale and the degree of environmental differentiation between populations. Local adaptation was negatively related to Source population size for two traits (seedling survivorship and the number of leaves at 12 months), indicating that augmenting with small Source populations may result in increased apparent local adaptation due to the introduction of inbred individuals with reduced fitness compared to the local Target population.

To examine outbreeding depression, F_1, F_2, F_3 and control (within Target population) progeny were generated for 12 of the 18 population pairs using a glasshouse crossing experiment. For the second and third generations, backcrosses to the Target and Source populations were generated to examine the two primary genetic mechanisms underlying outbreeding depression: (i) the dilution of genes associated with local adaptation (admixture analysis), and (ii) the disruption of co-adapted gene complexes through recombination (recombination analysis). F_1, F_2, F_3, backcrosses and control progeny were planted into soil from the Target population sites and, as for the local adaptation experiment, grown in a common climate representative of the Target populations. Germination and seedling survivorship as well as vegetative growth and reproduction

were measured at the juvenile (4 months), adult (8 months) and reproductive adult (12 months) life stages. Differences between the control (within Target population cross) and the F_1, F_2, F_3 and backcrosses for these fitness-related traits at the three life-stages (juvenile, adult and reproductive adult) were then compared for the 12 population pairs individually and in an overall analysis including all population pairs.

For the majority of individual population pairs and for the overall analysis, the fitness of inter-population hybrids was either equal to or greater than the local Target population for a range of fitness traits across the life cycle. There was some variability, however, in the fitness effects of inter-population hybridisation among different population pairs as well as across generations and between fitness traits, with greatest heterosis in the F_2 and backcross generations for germination, juvenile growth and cumulative fitness. These results indicate that inter-population hybridisation may potentially benefit long-term population viability and that fitness benefits may accumulate through the life cycle, even when outcrossing over large spatial scales of up to 600 km. Target and Source population size explained between 20 – 64 % of the variation in hybrid progeny fitness in the F_1, F_2 and backcross generations for a range of traits across the life cycle, with greatest heterosis in population pairs with small Target and large Source populations. This increase in fitness in small Target populations likely reflects increased genetic load and bi-parental inbreeding in small populations of *R. leptorrhynchoides*, while greater heterosis in population pairs with large Source populations signifies the importance of genetic diversity to hybrid progeny fitness and suggests that drift may play an important role in determining the genetic architecture of small populations.

Analysis of the fitness effects of admixture (diluting genes associated with local adaptation) and recombination (disruption of favourable epistasis) suggest that the overall fitness outcomes of inter-population hybridisation for *R. leptorrhynchoides* are determined by the complex interplay of a number of genetic mechanisms representing both additive and non-additive modes of gene action. These mechanisms also had differential effects on progeny fitness across the life cycle with the predominance of maternal boosting (through the transfer of heterosis from the F_1 to subsequent generations) in seedling and juvenile traits, while for later life history traits, the breakdown of co-adaptation led to reduced progeny fitness in some population pairs.

Evidence of both local adaptation and foreign gene advantage in the admixture analysis, and the potential for the stochastic creation of favourable gene combinations through recombination in later generations, suggests that these mechanisms may also contribute to progeny fitness following inter-population hybridisation.

To assess the relationship between fertilisation success and population size, within Target (control) and between (F_1, Target *x* Source) population crosses from the 12 population pairs in the outbreeding depression experiment were combined with control (within Target population) and F_1 (Target *x* Source) crosses from an additional 12 diploid and 5 tetraploid population pairs (24 diploid and 5 tetraploid pairs in total). There was a significant decrease in fertilisation success with declining population size for diploid and tetraploid populations of *R. leptorrhynchoides*, indicating the loss of *S*-alleles in small populations. Fertilisation 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 fertilisation success from crossing between populations. These results suggest that for small populations that have reduced fertilisation 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.

Taken together, the results of this research suggest that rather than a trade-off with the loss of local adaptation and outbreeding depression, inter-population hybridisation is likely to result in a two-fold benefit for small *R. leptorrhynchoides* populations; firstly through the restoration of *S*-allele diversity and secondly through increased fitness due to heterosis.

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1.1.1 Habitat fragmentation and population viability

Habitat fragmentation is a prominent threat to the maintenance of biodiversity, and is well recognised as a central issue in conservation biology (Hobbs & Yates 2003; Young & Clarke 2000). For species that were once widespread, habitat fragmentation can lead to a decline in the size and number of populations, and an increase in the spatial isolation of remnants. This alteration in population characteristics can have important implications for the long-term viability of small remnant plant populations due to changes in both genetic and demographic processes. Genetic factors in small populations that can affect population viability include bottlenecks and genetic drift (Soule 1987), changes in patterns of gene flow (Young et al. 1996), mutational meltdown (Lynch et al. 1995) and inbreeding depression (Dudash & Fenster 2000; Keller & Waller 2002). Demographic factors that can impact the viability of remnant populations include Allee effects (Oostermeijer et al. 2003), flowering asynchrony, pollinator limitation (Agren 1996; Kearns et al. 1998) and demographic and environmental stochasticity (Hobbs & Yates 2003). Genetic and demographic processes do not act in isolation, but may interact or act synergistically in the context of ecological factors to determine the fate of small populations. Moreover, species may respond differently to fragmentation events, since the significance of each of these processes is dependent on life history characteristics such as life-form, mating system, pollination and seed dispersal syndrome and generation time.

1.1.2 Genetic issues in small populations

1.1.2.1 Genetic diversity

The maintenance of genetic diversity is important for conserving the evolutionary potential of populations and the ability of populations to respond to current selection pressures (Savolainen & Kuittinen 2000). The loss of genetic diversity in small

populations may be due to two processes; firstly, the initial genetic bottleneck at the time of fragmentation, and secondly the loss of alleles due to subsequent random genetic drift if population size remains small. In both cases the loss of genetic diversity is primarily due to the elimination of rare, low frequency alleles (e.g. Young et al. 2000b). The significance of genetic drift in determining the genetic structure of populations is inversely related to population size, such that chance events become proportionally more important as population size declines. Conversely, selection may offset the loss of alleles through drift for loci under balancing or directional selection (Sherwin & Moritz 2000). Genetic issues in small populations, such as the loss of genetic diversity, have often been viewed as less important than demographic processes in determining the immediate persistence of remnant populations. However, the loss of genetic diversity of loci directly relevant to survival or reproduction such as the major histocompatibility complex (MHC) conferring disease resistance in vertebrates (e.g. Arkush et al. 2002; Hedrick 2004) or the self-incompatibility locus (*S*-locus) in plants (e.g. DeMauro 1993; Young et al. 2000b) can have an immediate and significant influence on demographic outcomes. This highlights the importance of understanding genetic processes that interact with demography to determine the long-term viability of remnant populations. In addition, the conservation of quantitative genetic variation is an important consideration in small populations, as the majority of demographic characteristics relevant to population viability are quantitative traits under polygenic control (Sherwin & Moritz 2000).

1.1.2.2 Inbreeding depression

Reduced population size can result in an increase in inbreeding through selfing in self-compatible species and/or an increase in bi-parental inbreeding (mating between related individuals) (Dudash & Fenster 2000). In both cases, increased inbreeding may result in a reduction in survival, growth and reproduction in inbred offspring (inbreeding depression) (Falconer & Mackay 1996). This may be particularly important for naturally outcrossing species that carry a high genetic load (Charlesworth & Charlesworth 1987; Young et al. 1996). Two mechanisms may contribute to inbreeding depression; heterozygote advantage (overdominance), whereby a decrease in heterozygosity *per se* leads to an associated decline in fitness, and the expression of deleterious recessive alleles that become homozygous through inbreeding (dominance). Although both have

been demonstrated to be responsible for inbreeding depression, there is currently more empirical evidence for dominance-based inbreeding depression (Carr & Dudash 2003; Charlesworth & Charlesworth 1999). However, the role and importance of epistasis (interactive effects among loci) as a genetic mechanism involved in inbreeding depression is largely unknown (Carr & Dudash 2003). Inbreeding depression can play a critical role in determining the viability of small and isolated populations (Frankham 1995, 2003; Keller & Waller 2002). Furthermore, this complex genetic process does not act in isolation and may interact with environmental variability (e.g. Dudash 1990) and demography (e.g. Oostermeijer 2000; Ouborg & Van Treuren 1997) to determine the persistence of small populations.

1.2 Conservation biology and the management of small populations

The primary objective in the conservation and management of threatened plant species is the maintenance or enhancement of population viability as species extinction results from the culmination of local population losses. In many cases, the management of small or declining populations may require intervention to counteract genetic and/or demographic processes that reduce the viability of remnant populations. One means is to introduce new genetic material by supplementing the population with individuals or seed from larger populations (genetic rescue) (Ingvarsson 2001; Richards 2000). Genetic rescue can provide two potential benefits for small populations. Firstly genetic rescue may counteract the deleterious effects of inbreeding depression and increase mean population fitness through heterosis (Dudash & Fenster 2000; Hedrick & Kalinowski 2000). Secondly, for species with self-incompatibility systems, the introduction of individuals with novel incompatibility alleles (*S*-alleles) can improve reproductive success by increasing mate availability (DeMauro 1993). Despite these potential benefits, the transfer of genetic material between populations introduces the issues of outbreeding depression and the disruption of patterns of local adaptation. In the absence of empirical studies, the potential risk of outbreeding depression has led to a cautious approach in the management of small populations where populations are maintained as genetically and /or geographically distinct entities.

This thesis aims to address a significant gap in our understanding of the importance of outbreeding depression and local adaptation in fragmented plant populations using the

model species *Rutidosis leptorrhynchoides* (Asteraceae). An additional aim of this thesis is to examine the predictive power of population size as well as geographic and environmental distance matrices to explain patterns of outbreeding depression and local adaptation. These results will then be examined in the context of *S*-allele diversity and the potential benefits to population viability of introducing new *S*-alleles into small populations.

Section 1.3 will define outbreeding depression, explore the underlying genetic mechanisms and discuss the factors that may affect the expression of outbreeding depression and local adaptation. Sections 1.4 and 1.5 consider the existing empirical evidence for local adaptation and outbreeding depression and highlight gaps in our current understanding of these processes. The value and predictive power of geographic and environmental distance matrices will be discussed in Section 1.6. Section 1.7 outlines and explores the potential trade-off between the benefits of introducing new *S*-alleles into small populations to increase mate availability vs. the potential negative effects of outbreeding depression. Section 1.8 and outlines the aims and structure of this thesis.

1.3 Outbreeding depression

1.3.1 What is outbreeding depression?

Outbreeding depression can be defined as a reduction in the fitness of offspring from inter-population crosses (inter-population hybrids) relative to the fitness of the parental populations. It is important to define outbreeding depression as it is a term that has been used in a range of contexts to describe a decline in fitness when crosses are undertaken: (i) within a single population (e.g. Parker 1992; Quilichini et al. 2001), (ii) between individuals from geographically or genetically distinct populations of a single species (e.g. Fenster & Galloway 2000a; Keller et al. 2000), (iii) between sub-species (Montalvo & Ellstrand 2001), and (iv) between species through inter-specific hybridisation (Ellstrand & Elam 1993; Fritz et al. 2006). Although the genetic mechanisms underlying outbreeding depression may operate in all of the above, this thesis will focus on the second definition; the intra-specific fitness effects of crossing between geographically distinct populations relative to the fitness of the local parental

population. Examining the fitness consequences of intra-specific hybridisation at the inter-population level is the scale most relevant and appropriate to both the conservation and restoration of threatened plant species, and our understanding of the evolutionary processes involved in population divergence and speciation.

1.3.2 Why is outbreeding depression important?

The issue of outbreeding depression is of critical importance in the management and restoration of threatened plants species as it is central to decision making in relation to the value of mixing genetic material from different plant populations to increase population size, augment genetic diversity (particularly *S*-allele diversity for self-incompatible species), counteract inbreeding, or establish new populations (Fenster & Galloway 2000a; Moritz 1999). Currently with limited research on the significance and importance of outbreeding depression, many conservation managers have adopted a precautionary approach and avoided the movement of plant material between populations, despite the potential benefits. Moreover, many seed collection guidelines for plant restoration include very limited geographic boundaries for seed collection zones. This may compromise the viability of restored populations if all seed is collected from small, genetically depauperate populations that happen to be local, rather than from larger, more diverse populations that may be geographically further away. Without an understanding of the role and importance of outbreeding depression, it is difficult to accurately assess the most appropriate action in the management of small populations. Furthermore, in an evolutionary context, understanding outbreeding depression and the genetic mechanisms involved will contribute significantly to our understanding of the evolutionary processes of adaptation, population divergence and speciation.

1.3.3 Mechanisms behind outbreeding depression

Outbreeding depression is primarily determined by two genetic mechanisms that are not mutually exclusive (Lynch 1991). These are; (i) the loss of local adaptation due to the mixing of genomes from different populations that have evolved independently in response to local environmental selection pressures (Cena et al. 2006; Dudash & Fenster 2000), and (ii) the disruption of co-adapted gene complexes that have evolved in different populations (Lynch 1991). It is important, however, to note that the fitness response of a population to inter-population hybridisation can be the result of the

interplay of a number of genetic mechanisms that may interact to determine the overall fitness outcomes of inter-population hybridisation. The following section will outline these two primary mechanisms involved in the expression of outbreeding depression and will also consider the range of potential mechanisms that may contribute to the overall fitness of populations following inter-population hybridisation, including additive *x* additive epistasis, cytoplasmic *x* nuclear epistasis, underdominance, maternal boosting and bi-parental inbreeding (Figure 1.1).

1.3.3.1 Local adaptation

The dilution of genes associated with local adaptation may occur when each parental population is fixed for different alleles at a number of loci conferring adaptation to local environmental conditions. Inter-population hybrids will have on average only half of the local genes from either parent and therefore may have reduced fitness compared to either parent in the local parental environment (Fenster & Galloway 2000a; Montalvo & Ellstrand 2001). This has been termed the 'ecological' mechanism (cf. Montalvo & Ellstrand 2001) which, due to the genotype *x* environment interaction, cannot be detected in controlled greenhouse conditions (Keller et al. 2000), and is likely to increase with increasing environmental differences between the parental populations (Montalvo & Ellstrand 2001). A decline in fitness in inter-population hybrids due to the dilution of genes associated with local adaptation can be observed in the F_1 generation, but should decrease in magnitude over subsequent generations due to segregation and restoration of parental genotypes (Figure 1.1).

1.3.3.2 Co-adapted gene complexes

The second mechanism, the disruption of co-adapted gene complexes (or intrinsic co-adaptation), is due to population divergence in genetic architecture as a result of the evolutionary processes of selection and genetic drift (Montalvo & Ellstrand 2001) and has been termed the 'genetic' (Montalvo & Ellstrand 2001) or 'physiological' (Roff 1997) mechanism. In its original and most extreme context, the term co-adapted gene complex was used to describe a multi-locus haplotype that is protected from recombination, having developed in response to local selection pressures (Dobzhansky-Muller incompatibilities) (Dobzhansky 1950). Following this definition, co-adapted gene complexes are disrupted during hybridisation through modification of the

frequency and distribution of chiasmata (e.g. Coates & Shaw 1982), and as such represent a genetic interaction that may provide an important post-mating reproductive barrier between taxa (Burke et al. 1998; Coates & Shaw 1982; Fishman & Willis 2001). However, in the literature regarding intra-specific outbreeding depression the term co-adapted gene complex is used to describe epistatic interactions between loci that confer a fitness benefit, where the selective advantage of a particular allele depends on alleles present at other loci (Falconer & Mackay 1996). Recombination following inter-population hybridisation may disrupt associations between beneficial combinations of interacting alleles, resulting in a loss of fitness in the F_2 and subsequent generations (Dudash & Fenster 2000; Fenster & Galloway 2000a). As outlined in Figure 1.1, outbreeding depression as a consequence of recombination between two differently adapted parental genomes will occur initially in the F_2, but may increase in severity in the F_3 and further generations with ensuing recombination. Conversely, there is the potential for recovery of fitness in later generation recombinants (e.g. Erickson & Fenster 2006), and for recombination to contribute to adaptive evolution through the generation of novel genetic variation and the chance creation of advantageous gene combinations (Burke & Arnold 2001; Fenster & Galloway 2000b; Whitlock et al. 1995).

1.3.3.3 Additive x additive epistasis

Additive *x* additive epistasis may influence the F_1 fitness outcomes of inter-population hybridisation (Lynch 1991) and occurs when alleles at one locus determine the selective value of alleles at other loci. If there is differentiation between populations so that particular combinations of alleles across loci confer some selective advantage, then disrupting these through inter-population hybridisation may result in an associated decline in fitness in F_1 progeny. In this case, an allele may have a selective advantage in the genetic background of its own population, but may be negative in the genetic background of another population (Bradshaw et al. 2005; Wade 2002).

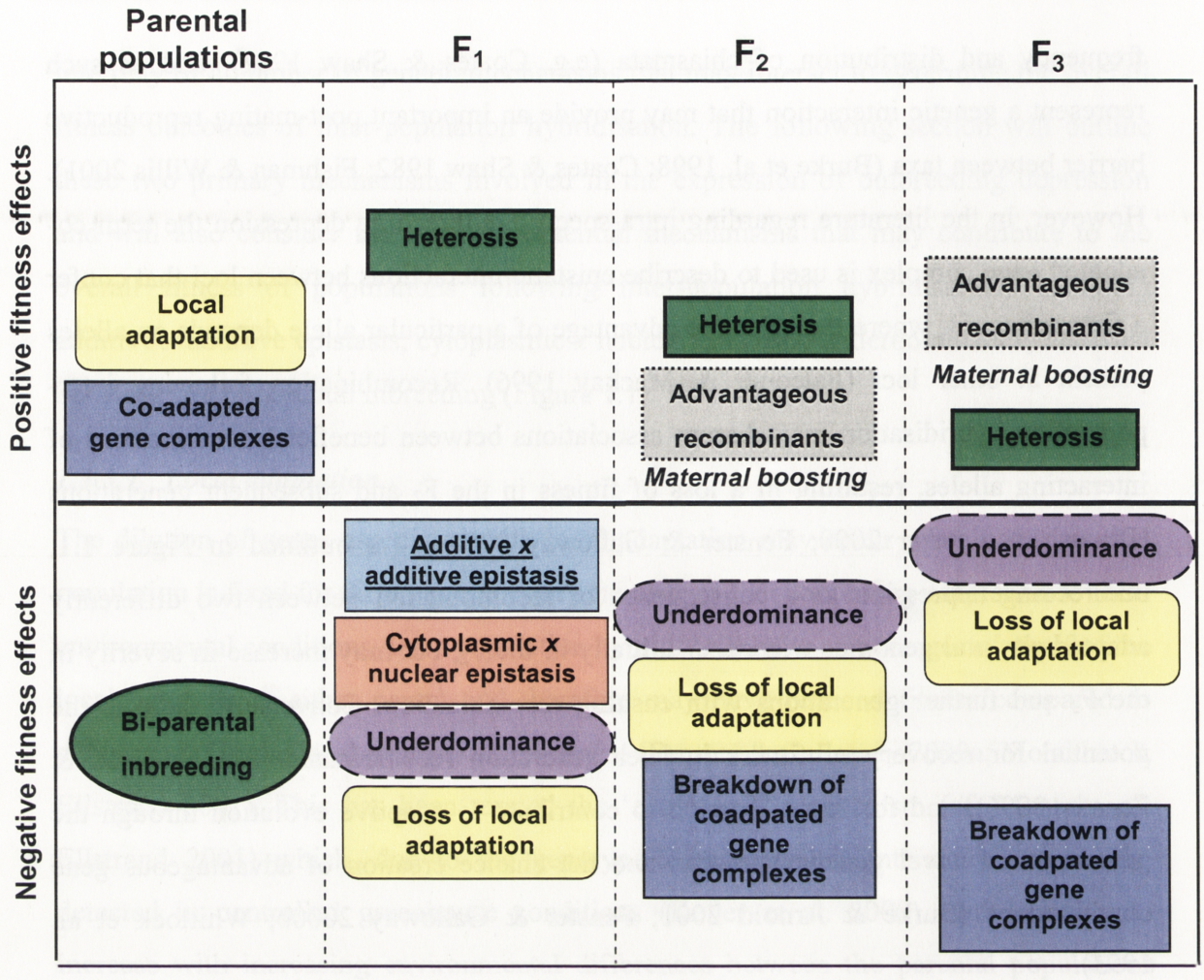

Figure 1.1 Potential genetic mechanisms that can influence fitness in the parental populations and the F_1, F_2 and F_3 following inter-population hybridisation.

Mechanisms above the solid line indicate positive fitness consequences, while those below the line indicate negative fitness consequences. The position above or below the line is not representative of the actual magnitude of the effect, however a change in the position of a mechanism in the F_1, F_2 or F_3 is indicative of the relative change in the potential importance of that mechanisms across generations.

1.3.3.4 Cytoplasmic-nuclear epistasis

The expression and magnitude of outbreeding depression may also depend on the cytoplasmic background on which the nuclear genes are expressed. The functional interaction between nuclear and cytoplasmic genes may contribute to population divergence such that when nuclear genes are expressed on a foreign cytoplasmic background there may be an associated decline in fitness due to disruption of positive epistasis between nuclear and cytoplasmic genes (Galloway & Etterson 2005). Due to the potential for cytoplasmic genes and cytoplasmic-nuclear epistasis to affect fitness and influence a population's response to selection (Galloway & Etterson 2005; Galloway & Fenster 1999; Galloway & Fenster 2001), the contribution of cytoplasmic and cytoplasmic-nuclear interactions to adaptive population differentiation needs to be considered when assessing the genetic effects of inter-population hybridisation. As shown in Figure 1.1, the disruption of cytoplasmic-nuclear epistasis may have a detrimental effect on fitness in the F_1 in hybrid offspring where nuclear genes are expressed on a foreign cytoplasmic background, although it is important to note that there can be temporal and environmental variation in the contribution of both nuclear and cytoplasmic genes to plant fitness (Galloway & Fenster 1999).

1.3.3.5 Underdominance

Underdominance occurs when heterozygotes are less fit than either homozygote (Whitlock et al. 1995). Since inter-population hybridisation between divergent populations will maximise heterozygosity in the F_1, underdominance can be the basis of reduced performance of inter-population hybrids (Galloway & Etterson 2005). Consequently, the magnitude of underdominance in response to inter-population hybridisation is predicted to increase with the degree of population differentiation (Galloway & Etterson 2005). The decline in fitness associated with underdominance will be greatest in the F_1, it is likely to decrease with subsequent generations due to segregation and a shift in the distribution of alleles back towards the parental genotypes (Figure 1.1).

1.3.3.6 Maternal boosting

In contrast to the detrimental effects on plant fitness of disrupting epistasis, a potential genetic mechanism that may result in increased fitness in the F_2 and F_3 following inter-

population hybridisation is 'maternal boosting', which is due to the relative difference in maternal effects experienced by the F_1 and F_2 generations. If the parental populations are mildly inbred then F_1 progeny from inter-population hybridisation would have higher fitness due to the elimination of bi-parental inbreeding. The F_2 generation are then produced in the superior maternal environment of the F_1 which translates to an increase in offspring fitness in the F_2. In the F_3, the effects of maternal boosting results in further increases in fitness for progeny produced in the enhanced maternal genetic environment of the F_2. Maternal effects are known to have a differential influence on fitness traits throughout the lifecycle (for reviews see Mousseau & Fox 1997; Roach & Wulff 1987) and accordingly, the contribution of maternal boosting to offspring fitness is likely to be greater in early life history traits (Byers 1998; Fenster 1991; Sobrevila 1988; Waser et al. 1995). Therefore, the relative importance of maternal boosting to the fitness response of inter-population hybrids could be tested by comparing fitness traits throughout the lifecycle for F_1, F_2 and F_3 offspring.

1.3.4 Hybrid vigour vs. hybrid breakdown: How do fitness effects vary across generations

The overall fitness response of a population to inter-population hybridisation will depend on the interplay of the potential genetic mechanisms discussed above, which may counteract one another, or act synergistically to determine mean population fitness, and which may change substantially between generations because of recombination (Figure 1.1). Due to the number of potential mechanisms involved in the expression of outbreeding depression, important questions arise such as: Can heterosis offset hybrid breakdown? And, what is the overall fitness response in the F_1, F_2 and F_3 progeny considering all potential genetic mechanisms?

1.3.4.1 F_1

The inbreeding history of a population can influence the magnitude of heterosis in the F_1 following inter-population hybridisation. Increased inbreeding in small, isolated populations with strong spatial structuring may result in inbreeding depression. As a consequence, inter-population hybridisation may result in a strong heterosis response in the F_1. Therefore, the overall fitness response in the F_1 will depend on the balance

between the positive effects of heterosis and the negative fitness effects associated with the loss of additive *x* additive epistasis, the dilution of genes associated with local adaptation, underdominance and any disruption of nuclear-cytoplasmic epistasis. In his model addressing the relative importance of inbreeding and outbreeding, Lynch (1991) suggests that for the F_1 to exhibit a decline in fitness relative to the parental populations the negative effects associated with the loss of favourable additive *x* additive epistasis would have to be twice as large as the benefit of heterosis, and concludes that the F_1 progeny of distant parents will always show enhanced fitness relative to progeny of parents from the same population. Consequently, when assessing the fitness effects of inter-population hybridisation, the interpretation of the F_1 performance will depend largely on the level of inbreeding in the parental populations (Rhode & Cruzan 2005).

1.3.4.2 F_2 and F_3

In the F_2 there are a number of possible scenarios for overall population fitness that depend on the potential for the loss of co-adapted gene complexes to counteract heterosis, the creation of advantageous recombinants and the flow-on effects of heterosis through maternal boosting. In addition, the loss of fitness associated with underdominance and the dilution of genes related to local adaptation is expected to be reduced in the F_2 due to a reduction in the frequency of heterozygous loci as a consequence of segregation. As has been suggested theoretically, this can result in the simultaneous occurrence of hybrid vigour and hybrid breakdown due to the concurrent action of heterosis, dilution of genes associated with local adaptation and the breakdown of co-adapted gene complexes (Lynch 1991), leading to an increase or a decrease in mean population fitness depending on the strength of each of these factors. The fitness response in the F_3 and subsequent generations will again depend on the relative magnitude of these factors such that although the influence of underdominance and local adaptation are expected to decline further, the break-down of co-adapted gene complexes may have a stronger effect in the F_3 than the F_2. Furthermore, the creation of advantageous gene combinations may occur in the F_3 through recombination, but this is predicted to increase the variance in fitness rather that the mean due to the chance creation of transgressive segregants.

In summary, the expression of outbreeding depression and/or heterosis across multiple generations is complex due to the interplay of a number of potential genetic mechanisms. The challenge in studying outbreeding depression empirically is to utilise a combination of experimental design and genetic tools to determine the relative importance and contribution of the various mechanisms over successive generations of inter-population hybridisation.

1.3.5 Factors that influence outbreeding depression

The primary factor that determines the level of outbreeding depression and/or heterosis following hybridisation is the degree of population divergence. A number of factors can influence population divergence such as mating system, gene flow, selection regimes, effective population size, and genetic drift. Moreover, each of these factors may affect outbreeding depression through the interaction with the different genetic mechanisms underlying the expression of outbreeding depression (see section 1.3.3). Although these factors will be discussed in isolation in the following section, it is essential to recognise the inter-relationships between these different factors and the importance of these interactions to population divergence, and through this, outbreeding depression.

1.3.5.1 Mating system

Theory predicts that mating system can influence the level of outbreeding depression in four ways. Firstly, any mating system that effectively reduces recombination, such as self-fertilisation (Glemin et al. 2006) will facilitate the formation of co-adapted gene complexes (Fenster et al. 1997; Templeton 1986). Secondly, increasing asexual reproduction or selfing will increase the likelihood of adaptation to local environmental conditions (Parker 1991; Dudash and Fenster 2000), suggesting that dilution of genes associated with local adaptation will be greater in asexual and selfing species. Thirdly, through promoting outbreeding, self-incompatible (SI) species may show less population differentiation than self-compatible species (Glemin et al. 2006; Linhart & Grant 1996), suggesting a lower risk of outbreeding depression in SI species (Dudash & Fenster 2000). Interestingly, in their model examining the effects of mating system on the magnitude and duration of outbreeding depression, Edmands & Timmerman (2003) found that partial self-fertilisation increased population divergence and the duration of outbreeding depression, but that the magnitude of outbreeding depression was less

compared to purely outcrossing species. However, in this model the fitness trajectory driven by local adaptation was very different to that of intrinsic co-adaptation, suggesting that the influence of mating system and other factors on the expression of outbreeding depression will depend on the mechanisms underlying the fitness effects of inter-population hybridisation.

The final means by which mating system may influence outbreeding depression is that it can affect the level of inbreeding in a population and therefore the potential for inbreeding depression and heterosis following inter-population hybridisation. Selfing species are predicted to show reduced heterosis since a history of inbreeding may purge genetic load and reduce inbreeding depression (Lande & Schemske 1985). It should be noted, however, that empirical studies have shown that purging is not a universal process and in many cases is an inconsistent force in reducing inbreeding depression (Boakes et al. 2007; Byers & Waller 1999; Frankham et al. 2001). In addition, the efficiency of purging and the magnitude of inbreeding depression may also be dependent on environment (Keller & Waller 2002), with some empirical studies suggesting that stressful environmental conditions may result in the more efficient purging of genetic load (e.g. Armbruster & Reed 2005; Reed et al. 2003; Swindell & Bouzat 2006).

1.3.5.2 Selection regimes

The magnitude and direction of selection as well as spatial and temporal variability in selection regimes can have an important influence on the potential for outbreeding depression. The role of environmental heterogeneity and local selection regimes in influencing patterns of genetic variation is well established empirically (Hedrick et al. 1976; Linhart & Grant 1996). The underlying mechanism that drives the association between environmental and genetic variation is that environmental heterogeneity can generate differentiation via selection. In this case, different environments generate different selection pressures that lead to population differentiation to local conditions. In addition, environmental heterogeneity can create barriers to gene flow which can enhance genetic differentiation among isolated populations (Linhart & Grant 1996). In contrast to spatial environmental heterogeneity that favours the development of local adaptation, temporal variation in selection regimes may act against the formation of

local adaptation by favouring generalist genotypes (Kawecki & Ebert 2004). Therefore the balance between spatial and temporal variability in selection regimes has important implications for the development of patterns of local adaptation and the potential for outbreeding depression.

The existence of population divergence and adaptation to local environmental conditions despite gene flow suggests that the strength of selection imposed by particular environmental conditions in local populations is stronger than the homogenising effects of gene flow (Kawecki & Ebert 2004; Sambatti & Rice 2006), although in many cases it is the combination of limited gene dispersal and environmental heterogeneity that results in local adaptation (Waser 1993; Waser et al. 2000). Moreover, due to the strength and importance of local selection as an evolutionary process in population divergence (Slatkin 1987), it has been suggested that local adaptation may evolve more rapidly than intrinsic co-adaptation which is driven by indirect selection and drift (Edmands & Timmerman 2003; Hendry et al. 2000; Waser & Williams 2001).

1.3.5.3 Gene flow

Gene flow is an important evolutionary force that may counteract population divergence and constrain local adaptation through the transfer of genetic material among populations (Lenormand 2002; Slatkin 1987). This can result in an interaction between population differentiation due to natural selection and the homogenising effect of gene flow (Kawecki & Ebert 2004). The complex interplay between spatially divergent selection and gene flow, and its effect on adaptive evolution, is well established both theoretically (Haldane 1930; Slatkin 1987) and empirically (Kawecki & Ebert 2004; Linhart & Grant 1996; Sambatti & Rice 2006; Storfer 1999). Even though considerable temporal and seasonal variation in the level of gene flow has been found within and between species (see Ellstrand 1992b), the level of gene flow observed in many species may be sufficient to counteract moderate levels of spatially divergent selection and drift (Ellstrand 1992a), leading to a reduced likelihood of outbreeding depression through the disruption of local adaptation. However, gene flow may result in different outcomes depending on whether it is mediated by seed dispersal, pollen dispersal, or both. Specifically, as cytoplasmic factors are transmitted in seed but not pollen, differential

dispersal of pollen and seed may lead to distinctive patterns of differentiation for nuclear compared to cytoplasmic genes as well as population divergence for cyto-nuclear epistasis (Galloway & Fenster 1999). The homogenising effects of gene flow may also potentially counteract the development of co-adapted gene complexes, yet there is evidence that unique co-adapted gene complexes can occur in separate populations even in the presence of gene flow (Endler 1977).

It is also important to consider effective gene flow between populations, that is, the incorporation of migrants into the new population. For example, in self-incompatible species negative frequency dependent selection will facilitate the efficient migration of individuals with novel *S*-alleles into a new population as migrants with rare or novel *S*-alleles will have a high selective advantage (Castric & Vekemans 2004; Schierup et al. 2000). This selection for fertilisation success may facilitate the spread and incorporation of foreign genes into the population and in doing so counteract the development of local adaptation and/or the formation of co-adapted gene complexes. This may be especially important in small populations that are limited by low number of *S*-alleles. Moreover, in inbred populations there may be amplification in the rate of gene flow due to hybrid vigour (Ingvarsson & Michael 2000). In this case, genotypes from immigrants may increase and spread through the population because they carry a fitness advantage through heterosis (e.g. Ebert et al. 2002; Saccheri & Brakefield 2002).

1.3.5.4 Effective population size

Effective population size plays a pivotal role in determining the relative importance of drift vs. selection to genetic architecture and population differentiation (Sherwin & Moritz 2000). For small and isolated populations, population bottlenecks and genetic drift will have a dominant influence on population genetic structure. In contrast, for large populations, selection is the principal factor that structures genetic variation (Barrett & Kohn 1991; Linhart & Grant 1996). This has important implications for population divergence and the potential for outbreeding depression because genetic drift and selection can have a differential influence on the various mechanisms underlying outbreeding depression. These processes will also interact with other factors such as mating system and gene flow, since selfing will reduce effective population size (Glemin et al. 2006). Moreover, the level of gene flow and the strength of selection are

important factors that contribute to the balance between drift and selection in determining patterns of population divergence.

Small effective population size (often in combination with fine scale genetic structure, inbreeding and limited gene dispersal) will favour restricted recombination and facilitate the development of co-adapted gene complexes (Barrett & Kohn 1991; Templeton 1986). In contrast, local adaptation may be lower in small populations due to the reduced effectiveness of selection relative to genetic drift (Ellstrand & Elam 1993; Glemin et al. 2006; Hedrick & Miller 1992). These contrasting processes suggest that in small populations there may be a higher risk of disrupting intrinsic co-adaptation, but a lower risk of outbreeding depression through dilution of genes associated with local adaptation. Furthermore, the reduced efficacy of selection to remove deleterious alleles when effective population size is small may lead to greater inbreeding depression (Ellstrand & Elam 1993) and therefore result in a strong heterosis response following inter-population hybridisation. Again this demonstrates that the influence of various factors on the expression of outbreeding depression and/or heterosis will depend on the interplay of each factor with the underlying genetic mechanisms.

1.3.5.5 Ploidy level (cytological variation)

Variation in ploidy level may theoretically have an important influence on the expression of outbreeding depression, although no empirical studies have directly examined outbreeding depression in relation to ploidy level. There are five primary theoretical expectations regarding the potential influence of ploidy level on outbreeding depression which predict different outcomes following inter-population hybridisation:

1. Theoretical models suggest that polyploids may show a greater rate of adaptation as an increase in the number of copies of alleles will increase the chance of mutation and decrease the strength of selection on any particular allele (Otto & Whitton 2000). Moreover, allelic redundancy allows for the possibility of alleles acquiring novel functions (Otto & Whitton 2000). These ideas of greater adaptability in polyploids can also be linked to the concept of the adaptive landscape (cf. Wright 1931; Wright 1969). In this case, polyploids may occupy a different position in the adaptive landscape (Wright 1969) or be able to

move across adaptive landscapes more rapidly than diploids, which may translate into a faster rate of evolution in polyploids compared to diploids. This potential increase in the rate of evolution in polyploids may lead to higher levels of population divergence, co-adaptation and/or greater local adaptation among polyploid populations, thereby suggesting an increased risk of outbreeding depression.

2. Having multiple copies of a genome means that polyploids have more genes and hence greater opportunity for interactions between genes to contribute to genetic variation. This may lead to potentially higher levels of intrinsic co-adaptation and thus a greater chance of disrupting co-adapted gene complexes through inter-population hybridisation for polyploid compared to diploid populations (Dudash & Fenster 2000).

3. Polyploidy may modify rates of recombination compared to diploids due to differences in chromosome alignment during meiosis. For example, it has been demonstrated for *R. leptorrhynchoides* that diploids had a higher chiasma frequency per chromosome (0.90) compared to tetraploids (0.77) (Murray & Young 2001). This potential for lower recombination rates in polyploids may promote the evolution of co-adapted gene complexes and thereby increase the risk of outbreeding depression in polyploid populations through the disruption of intrinsic co-adaptation.

4. Population differentiation may be greater in polyploids compared to diploids as multiple genomes allow for the functional divergence of gene copies (Galloway & Etterson 2005). Consequently greater outbreeding depression may be expected for polyploids. Population differentiation and the contribution of cyto-nuclear epsistasis to the expression of outbreeding depression may also differ between diploids and autoployploids because autopolyploids have a different ratio of cytoplasmic to nuclear genes compared to diploids (Galloway & Etterson 2005).

5. Increases in ploidy level can provide a selective advantage by masking the deleterious fitness effects of mutations and thereby reduce mutation load (Otto & Whitton 2000). This, coupled with the maintenance of higher genetic diversity in ploypoids (Bever & Felber 1992) and the existence of partial heterozygotes (Husband & Schemske 1997), may result in reduced inbreeding depression in ploypoid compared to diploid populations, which has been observed empirically (Buza et al. 2000; Husband & Schemske 1997). This has important implications for the heterosis response following inter-population hybridisation and suggests that the fitness benefits of crossing between populations may be less in polyploid populations due the reduced potential for heterosis.

1.4 Local adaptation: empirical evidence

Local adaptation is the process of adaptive genetic differentiation among populations in response to local selection pressures. As discussed in section 1.3, adaptive genetic differentiation among populations can be due to the action of three processes that may act synergistically and/or interact to determine the level of divergence between populations. These processes are: i) selection across spatially heterogeneous environments, ii) restricted gene flow between populations, and iii) genetic drift. Patterns of local adaptation have long been the subject of empirical research (e.g. Turesson 1922) and recent studies have highlighted the importance of empirical tests of local adaptation to plant restoration and conservation (Galloway & Fenster 2000; Gordon & Rice 1998; Hufford & Mazer 2003; Montalvo & Ellstrand 2000).

The results of local adaptation experiments, however, tend to vary, with some studies finding strong local adaptation (e.g. Kittelson & Maron 2001; Nagy & Rice 1997; Sambatti & Rice 2006; Schmidt & Levin 1985), and others little (e.g. Galloway & Fenster 2000; Jakobsson & Dinnetz 2005) or no evidence of local adaptation (e.g. Gordon & Rice 1998; Helenurm 1998; Rapson & Wilson 1988). Moreover, despite the expected relationship between spatial scale and local adaptation due to the predicted increase in both degree of environmental differentiation and genetic isolation with geographic distance (Galloway & Fenster 2000; Linhart & Grant 1996), the generality of this relationship has not been established. Two recent studies found a weak

relationship between local adaptation and geographic distance between populations (Galloway & Fenster 2000; Montalvo & Ellstrand 2000). In contrast, Becker et al. (2006a) found a significant negative relationship between plant fitness and the geographic distance between the home and transplant site. Yet in this study, the climatic variables that drive local adaptation were highly correlated with geographic distance. This highlights the importance of spatial patterns of environmental heterogeneity to the relationship between adaptive differentiation and spatial scale. This is substantiated by a study of the subshrub *Lotus scoparius* where, compared to geographic distance, genetic and environmental distance had greater power to predict patterns of local adaptation (Montalvo & Ellstrand 2000), suggesting a more complex spatial pattern of environmental heterogeneity. Theory also suggests that mating system, spatial and temporal environmental heterogeneity and life history traits such as longevity should have an important influence on the expression of local adaptation (see section 1.3).

A recent meta-analysis of 35 local adaptation studies, which included 1032 pair-wise comparisons of the performance of local and foreign plants, found consistent evidence for local adaptation (Leimu & Fischer in review). Despite theoretical expectations, however, mating system, spatial and temporal environmental heterogeneity, plant longevity and clonality had little influence on patterns of local adaptation. Instead population size was found to be the key factor determining local adaptation, with local adaptation found in large, but not small populations (Leimu & Fischer in review). The relationship between local adaptation and population size may be attributed to three main factors: (i) the relative importance of selection and drift change with population size (see section 1.3.5.4), (ii) a reduced likelihood of the stochastic creation of beneficial mutations in small populations (Leimu & Fischer in review) and (iii) the homogenising effects of gene flow that constrain local adaptation may be greater in small populations compared to large ones, since constant gene flow will have a greater effect when population size is small (Holt & Gomulkiewicz 1997; Jakobsson & Dinnetz 2005).

Across all studies, local adaptation was not significantly associated with geographic distance between the populations, but at shorter distances the variation in local adaptation was much greater compared to larger spatial scales (Leimu & Fischer in

review). This suggests that at small scales environmental heterogeneity can be large enough to result in local adaptation, but that this is less consistent than at large spatial scales. It is also important to note that this pattern will depend on the interaction of gene flow and selection, since the relationship between these two evolutionary forces is likely to change with geographic distance between populations (Linhart & Grant 1996). At smaller spatial scales stronger selection may be required to overcome the homogenising effect of gene flow and allow the development of local adaptation (Kawecki & Ebert 2004; Lenormand 2002; McKay & Latta 2002). The evolutionary significance of the interaction between gene flow and selection was examined in a recent study of the Californian Sunflower *Helianthus exilis*. In this study, strong selection maintained adaptive differentiation despite extensive gene flow between populations, which suggests that although gene flow was unable to counteract local adaptation, it prevented local speciation between ecotypes (Sambatti & Rice 2006).

1.5 Outbreeding depression: empirical evidence

Despite the potential importance of outbreeding depression to conservation biology and our understanding of the processes of population divergence and speciation, there are still relatively few studies that have addressed this issue empirically (see Edmands 2007). Since the focus of this thesis is on outbreeding depression in fragmented plant populations, a review of current empirical research will focus predominantly on the plant literature. However, there are important parallels between studies of outbreeding depression in plant and animal systems because both the underlying genetic mechanisms, and the factors that influence population divergence, are analogous in both plant and animal taxa. This is highlighted in a recent review of empirical studies of inbreeding and outbreeding across a range of taxa (Edmands 2007). Using the small number of studies that included a comparison of the fitness effects of inbreeding and outbreeding, Edmands (2007) found that the risk of inbreeding exceeded the risk of outbreeding in the F_1, but that in the F_2, there was a similar risk of inbreeding and outbreeding. This conclusion suggests that the risk of outbreeding depression may be comparable to inbreeding depression, but due to the lack of empirical studies is far from conclusive.

1.5.1 Optimal outcrossing and outbreeding depression

In examining the relationship between progeny fitness and the spatial scale of the parental plants, it is important to distinguish between the *intra*-population optimal outcrossing literature and the literature dealing with *inter*-population hybridisation. Optimal outcrossing has been described in a number of plant species (see Sobrevila 1988; Waser 1993 and references therein; Waser & Price 1983) and focusses on the fitness consequences for progeny of crosses within or between local demes and relates to detecting an optimal outcrossing distance to avoid the detrimental effects of inbreeding and outbreeding depression within a population. Optimal outbreeding has been predominantly found in species with fine scale genetic structure, limited gene dispersal and fine scale spatial heterogeneity in selection regimes (Quilichini et al. 2001; Waser et al. 2000), although many empirical studies have detected significant environmental and temporal variation in the expression of optimal outcrossing distances (Waser et al. 2000). Optimal outcrossing has interesting implications for understanding the evolutionary dynamics of mating patterns within a population. However, as addressed in this thesis, examining the fitness consequences of inter-population hybridisation over broad spatial scales relevant to species distributions is the scale directly relevant to plant conservation and restoration as well as the evolutionary processes of population divergence and speciation.

1.5.2 Outbreeding depression: The plant literature

Empirical studies that have examined the issue of outbreeding depression and the fitness consequences of inter-population hybridisation in plant species are outlined in Table 1.1. This table summarises the 24 studies of 27 plant species that have examined outbreeding depression and/or heterosis through inter-population hybridisation. An examination of the literature highlights five important issues in relation to our current understanding of outbreeding depression, the gaps in the literature and future research directions. Each of these issues will be discussed in the following section.

1.5.2.1 The fitness of inter-population hybrids beyond the F_1

An examination of the current outbreeding depression literature highlights the scarcity of studies that have examined the fitness consequences of inter-population hybridisation beyond the F_1 (Table 1). Currently, the majority (83 %) of studies have only undertaken

F_1 crosses between parental populations. As discussed in section 1.3.3, an assessment of the fitness of F_1 progeny will only allow examination of the mechanisms of local adaptation, underdominance and loss of additive *x* additive epistasis, not hybrid break-down through the disruption of co-adapted gene complexes. This means that the overall fitness response to inter-population hybridisation may be driven by heterosis, which is strongest in the F_1. In addition, approx 80 % of the studies that have examined the fitness of F_1 hybrids were undertaken in greenhouse conditions and/or a single common garden where local adaptation to specific environmental conditions cannot be tested due to the genotype *x* environment interaction. Therefore, our current understanding of the fitness consequences of inter-population hybridisation over the first, second and third generations comes from the results of three key studies that have examined the fitness of inter-population hybrids beyond the F_1 in a field environment (Erickson & Fenster 2006; Fenster & Galloway 2000a, b; Keller et al. 2000). These results are discussed below.

The issue of outbreeding depression in relation to spatial scale and epistasis has been well studied in natural environments in the self-compatible, but highly outcrossing annual legume *Chamaecrista fasciculata* (Erickson & Fenster 2006; Fenster & Galloway 2000a, b). In these studies, heterosis was consistently found in the F_1, even at the longest distance classes (100 – 2000km), suggesting that populations are inbred and that the masking of deleterious recessive through inter-population crosses resulted in an increase in fitness in F_1 progeny (Fenster & Galloway 2000a, b). The fitness of F_2 hybrids was intermediate between the parents and F_1, but varied between fitness traits and across different distance classes. This intermediate fitness response suggests a decline in heterosis in the F_2, but no associated break-down of co-adapted gene complexes. In the F_3 there was more consistent evidence for the disruption of co-adapted gene complexes and loss of heterozygosity, yet for some fitness traits the performance of the F_3 progeny was similar to the composite generation (an average of the parental populations and F_1) and in a few cases showed positive epistasis (Dudash & Fenster 2000; Fenster & Galloway 2000a, b). It was only at the longer distance classes that break-down of co-adapted gene complexes outweighed the positive effects of heterosis, resulting in the expression of outbreeding depression. Further experiments

with *C. fasciculata* to examine the potential for recovery of fitness in later generation recombinants examined the performance of recombinant F_6 and F_2 generations compared to the composite generation. Interestingly, this study found that lifetime fitness (survivorship *x* reproduction) and biomass production showed strong recovery in the F_6 and that for biomass production the F_6 performed significantly better than the F_2. Germination was the only exception where the F_6 performed significantly below the composite generation (Erickson & Fenster 2006). These results suggest that even if outbreeding depression is apparent in early generation hybrids due to the breakdown of co-adapted gene complexes, recombination in subsequent generations may result in recovery of, and/or an increase in, mean plant fitness.

Keller et al. (2000) examined the fitness of F_1 and F_2 inter-population hybrids in three weed species *Agrostemma githago*, *Papaver rhoeas*, and *Silene alba* compared to the performance of parental populations. Differences in the fitness effects of inter-population hybridisation were detected between generations for the three species. For *P. rhoeas* and *A. githago* outbreeding depression due to the disruption of epistasis was detected in the F_2 for plant biomass in both species and for survivorship in *P. rhoeas*. In contrast, biomass was not reduced in the F_2 for *S. alba*, and instead heterosis for seed mass was detected in the F_1, but subsequently decreased in the F_2. Differences in the fitness of inter-population hybrids across generations in these three species reiterate the importance of examining the fitness effects of inter-population hybridisation across multiple generations.

1.5.2.2 Variability in outbreeding depression across populations, environments and fitness traits

An examination of the current literature reveals substantial variation in the fitness of inter-population hybrids between population comparisons, experimental sites and years, as well as across different fitness traits. This variability in the expression of outbreeding depression has important implications for both the interpretation of experimental results

Table 1.1 (on following page) A comparison of empirical studies that have investigated the fitness consequences of inter-population hybridisation

[1]Mating system: SC = Self-compatible, SI = Self-incompatbile and D = Dioecous. [2]Spatial scale of inter-population crosses. [3]Control or comparison for the fitness of inter-population hybrids (as fitness is a relative measure of the performance of different genotypes). [4]Fitness response: + (heterosis), - (outbreeding depression), = (equivalent performance of inter-population hybrids and control). [5]G = Glasshouse, F = Field and CG = Common Garden. [*]Year to year variation in response. [‡] Site to site variation in response. [α]Response dependant on fitness trait measured. [ß]Response differed between population comparisons. [γ]Response varied with population size. [δ]Trend for a decrease in fitness, but not statistically significant. [a]Average of the two parental populations. [b]Composite generation is the average of the two parental populations and the F_1 and is used to quantify epistasis

Species	Mating system[1]	Spatial scale[2]	Control[3]	Fitness Response[4] F_1	F_2	F_3	F_6	G, F or CG[5]	No. target populations	Reference
Chamaecrista fasciculata	SC	0.1-2000 km	Home and away parents	$+^{*\ddagger}$		=		F	3	Fenster & Galloway (2000)a
Chamaecrista fasciculata	SC	0.1-2000 km	Mid-parent value for F_1[a], composite generation for F_2 and F_3[b]	$+/=^{*\ddagger\alpha}$	$+/=/-^{*\ddagger\alpha}$	$+/=/-^{*\ddagger\alpha}$		F	3	Fenster & Galloway (2000)b
Chamaecrista fasciculata	SC	2000 km	Home and away parents	$+/=^{*\ddagger}$	$+/=^{*\ddagger}$		$+/=^{*\ddagger}$	F	2	Erickson & Fenster (2006)
Camplanula americna	SC	1.5-550 km	Average of parental populations	$+/=/-^{\alpha\beta}$				G	2	Galloway & Etterson (2005)
Lotus scoparius var *brevialatus* and *Lotus scoparius* var *scoparius*	SC	60-340 km	Fitness correlated with genetic and environmental distance between populations	-				G	6	Montalvo & Ellstrand (2001)
Lotus scoparius var *brevialatus* and *Lotus scoparius* var *scoparius*	SC	60-340 km	Fitness correlated with genetic and environmental distance between populations	=/-				CG	6	Montalvo & Ellstrand (2001)
Agrostemma githago	SC		Home and away parents	=	-			F	1	Keller et al. (2000)
Papaver rhoeas	SI		Home and away parents	$=^{\delta}$	$=/-^{\alpha\beta}$			F	1	Keller et al. (2000)
Silene alba	D	up to 1000 km	Home and away parents	$+/=^{\beta\delta}$	$=/-^{\alpha}$			F	1	Keller et al. (2000)
Dalechampia scandens	SC	Within regions and between regions	Parental populations	$=/-^{\alpha\beta}$				G	4	Pelabon et al. (2005)
Espeletia schultzii	SI	up to 78 km	Parental populations	=/-				NA	1	Sobrevila (1988)
Eupatorium resinosum	SI	30 km	Crosses between near (1.5 m) and far (15-25 m) individuals within a population	+				CG	1	Byers (1998)
Eupatorium perfoliatum	SI	30 km	Crosses between near (1.5 m) and far (15-25 m) individuals within a population	+				CG	2	Byers (1998)
Leavenworthia alabamica	SI/SC	up to 80 km	Within population cross	$+/=/-^{\alpha\beta}$				G	8	Busch (2006)
Eucalyptus globulus ssp *globulus*	SC	21-100 km	Open pollinated offspring and selfs	=				F	1	Hardner et al. (1998)
Silene vulgaris	SC	1-500 km	Self-pollination and within population cross	$+/=/-^{\alpha\beta}$	=			G	3	Bailey & McCauley (2006)
Arnica montana	SI		Self-pollination and within population cross	$+/=^{\beta}$				F	5	Luijten et al. (2002)
Hypochoeris radicata	SI	43-650 km	Self-pollination, within-family and within population cross	$=/-^{\alpha\beta}$				G/CG	3	Becker et al. (2006)
Viola pumila	SC		Self-pollination and within population cross	=				CG	2	Eckstein & Otte (2005)
Viola stagnina	SC		Self-pollination and within population cross	=/-				CG	2	Eckstein & Otte (2005)
Banksia ilicifolia	SC	30 km	Self-pollination and within population cross	+				G	1	Heliyanto et al. (2006)
Calylophus serrulatus	SC	20 km	Self-pollination and within population cross	$=/-^{\alpha\beta}$				G	3	Heiser & Shaw (2006)
Syzgium rubicundum	SC	12 km	Self-pollination and within population cross	$=/-^{\alpha}$				G	1	Stacy (2001)
Shorea cordifolia	SI	35 km	Self-pollination and within population cross	$=/-^{\alpha}$				G	1	Stacy (2001)
Gentiana pneumonanthe	SC	800 m	Self-pollination and within population cross	+				G	1	Oostermeijer et al. (1995)
Gentiana germanica	SC	25 km	Self-pollination and within population cross	$=/-^{\alpha}$				G/CG	1	Fisher & Matties (1997)
Gentiana germanica	SC		Self-pollination and within population cross	$+/=/-^{\alpha\gamma}$				CG	12	Paland & Schmid (2003)
Sarracenia flava	SC	Up to 30 km	Self-pollination and within population cross	+				G	6	Sheridan & Karowe (2000)
Silene nutans	SC	30 km	Self-pollination and within population cross	$+/=^{\beta}$				NA	2	Hauser & Seigismund (2000)
Scabiosa columbaria	SC	4-69 km	Self-pollination and within population cross	$+/=^{\alpha\beta}$				G	6	van Treuren et al. (1993)
Lychnis flos-cuculi	SC		Self-pollination and within population cross	$+/=^{\alpha\beta}$				G/CG	4	Hauser & Loeschcke (1994)

and predicting the long-term demographic consequences of inter-population hybridisation.

A number of studies of outbreeding depression that include multiple populations have detected significant variation in the fitness of inter-population hybrids between different population comparisons (e.g. Bailey & McCauley 2006; Becker et al. 2006b; Galloway & Etterson 2005; Hauser & Siegismund 2000; Heiser & Shaw 2006; Keller et al. 2000; Luijten et al. 2002; Pelabon et al. 2005). However, almost half (47 %) of the studies on outbreeding depression have only used 1 or 2 populations to assess the fitness consequences of inter-population hybridisation (Table 1). This variation between populations suggests that populations may respond quite differently to inter-population hybridisation and that particular characteristics (e.g. population size or history) may influence the expression of outbreeding depression. As a result, caution needs to be taken when the results from a limited number of populations are used to predict the consequences of inter-population hybridisation, since the results from one or two populations may not necessarily be representative of the species response.

The results of studies with replication across sites and years (e.g. for *Chamaecrista fasciculata*; Erickson & Fenster 2006; Fenster & Galloway 2000a, b) indicate that spatial and temporal variation in environments can influence the expression of outbreeding depression and highlight the importance of examining outbreeding depression in natural conditions. Furthermore, it is essential to recognise that studies undertaken at a single site and/or in a particular year provide an assessment of outbreeding depression in the context of a specific set of environmental conditions.

The basis of any study examining outbreeding depression is the relative difference in fitness of inter-population hybrids compared to the parental populations. Half of the studies that have examined the fitness consequences of inter-population hybridisation have detected variation in the expression of outbreeding depression across different fitness traits (e.g. Bailey & McCauley 2006; Becker et al. 2006b; Fenster & Galloway 2000b; Fischer & Matthies 1997; Galloway & Etterson 2005; Heiser & Shaw 2006; Keller et al. 2000; Pelabon et al. 2005; Stacy 2001) (Table 1). Considering this variation in the response of different fitness traits, knowing which traits have the greatest

demographic importance is critical to understanding the fitness outcomes of inter-population hybridisation and the potential effects on long-term population viability.

Current studies of outbreeding depression have used one or a combination of individual fitness traits (e.g. Keller et al. 2000; Luijten et al. 2002), cumulative fitness measures (e.g. Fenster & Galloway 2000b; Montalvo & Ellstrand 2001; Stacy 2001) and/or lifetime fitness (Erickson & Fenster 2006) to assess the performance of inter-population hybrids relative to the parental populations. No studies, however, have used elasticity analysis in combination with the response of different fitness traits to examine the demographic consequences of variation in fitness across the life-cycle. In elasticity analysis, individual fitness traits are weighted by their demographic importance and contribution to population viability (Caswell 2001) and enable the interpretation of fitness components in a demographic context (Crone 2001). Combining information on demography and the fitness response of particular traits across the life-cycle provides a promising means of assessing the potential outcomes of inter-population hybridisation for long-term population viability, and is especially important in light of the observed variation in the expression of outbreeding depression across different fitness traits.

1.5.2.3 Genetic mechanisms: Which is the most important?

As outlined in section 1.3.3, outbreeding depression results from the complex interaction of a number of genetic mechanisms. The relative importance of each of these genetic mechanisms is currently not well understood, mostly due to the limited number of empirical studies that have examined outbreeding depression beyond the F_1 in a field environment (Table 1.1). A few recent studies provide important contributions to our understanding of the different genetic mechanisms underlying outbreeding depression and the genetic basis of population divergence. These studies include assessments of; epistasis and the breakdown of co-adapted gene complexes (Bailey & McCauley 2006; Fenster & Galloway 2000b; Keller et al. 2000), cytoplasmic-nuclear epistasis (Galloway & Etterson 2005; Galloway & Fenster 1999; Pelabon et al. 2005) and local adaptation through a combination of transplant and crossing experiments (e.g. for *Chamaecrista fasciculata* (Fenster & Galloway 2000b; Galloway & Fenster 2000) and *Lotus scoparius* (Montalvo & Ellstrand 2000, 2001)). However more empirical studies are required that

explicitly test the relative importance and contribution of different genetic mechanisms to the expression of outbreeding depression, particularly over multiple generations.

1.5.2.4 Mating system

As outlined in section 1.3.5.1, mating system can have an important influence on the potential risk of outbreeding depression. Currently, 67 % of studies examining the fitness consequences of inter-population hybridisation have been undertaken on self-compatible (SC) species (Table 1). A comparison of outbreeding depression in closely related species with different mating systems would provide an ideal empirical framework in which to test the hypothesis of greater outbreeding depression in SC compared to self-incompatible (SI) species.

In his study of heterosis in the F_1 in large SI and small SC populations of *Leavenworthia alabamica*, Busch (2006) found little heterosis or outbreeding depression following inter-population hybridisation in five SI populations and two of the three SC populations. Substantial heterosis was only observed in one SC population, while significant outbreeding depression was detected in another SC population, although the fitness response for both heterosis and outbreeding depression varied across fitness traits. This implies that small population size and reproductive isolation in combination with self-compatibility may increase the potential for heterosis in the F_1 and that self-compatibility may increase the risk of outbreeding depression. However, the interpretation of these results is limited to the F_1 only. In an examination of outbreeding depression in the F_1 and F_2 of three species with different mating systems, Keller (2000) found some evidence for greater outbreeding depression in hybrid offspring of the SC species *A. githago*, although outbreeding depression was also detected in the SI species *P. rhoeas*. These results suggest that although SC species may be more sensitive to outbreeding depression, this is an issue that clearly requires further study.

1.5.2.5 Spatial scale

A number of studies of outbreeding depression have examined inter-population hybridisation over a range of spatial scales pertinent to species distributions (Bailey & McCauley 2006; Becker et al. 2006b; Fenster & Galloway 2000a, b; Galloway &

Etterson 2005; Keller et al. 2000) and the distances often considered for translocation and/or revegetation (Montalvo & Ellstrand 2001) (Table 1.1). However, to ascertain the spatial scale over which population divergence and outbreeding depression become important, more studies are required that include a range of spatial scales between parental populations that cover the entire geographic distribution of the species. Examination of outbreeding depression over these scales could facilitate the detection of thresholds above which the movement of genetic material between populations can have negative consequences for plant fitness (i.e. the delineation of appropriate seed transfer zones (see Hufford & Mazer 2003)). An understanding of these thresholds has very practical conservation implications, but is also interesting from an evolutionary perspective as it provides information on the spatial scale of population divergence, and ultimately, speciation.

1.5.2.6 Future research directions

Although a number of recent studies provide important examples of empirical testing of outbreeding depression and the fitness consequences of inter-population hybridisation, to bridge the gaps in our understanding of outbreeding depression more empirical studies are required that: (i) examine hybrid fitness over several generations, (ii) measure a range of fitness traits across the life-cycle under natural conditions, (iii) use multiple population comparisons, (iv) examine a range of spatial scales and (v) attempt to experimentally discriminate between the potential mechanisms underlying outbreeding depression. Information from these types of studies will help to elucidate the role and importance of outbreeding depression to the long-term viability of fragmented plant populations following augmentation and help to clarify the genetic basis of any fitness differences in inter-population hybrids across generations.

1.5.3 Outbreeding depression: The animal literature

In parallel with the plant literature, empirical studies examining outbreeding depression in animal taxa reflect both the complexity of the genetic mechanisms underlying outbreeding depression and the potential variability in the fitness consequences of inter-population hybridisation. Studies examining the fitness of inter-population hybrids in animal taxa have found evidence for heterosis (Coulson et al. 1998) and outbreeding depression (Burton 1987, 1990; Ellison & Burton 2006; Marshall & Spalton 2000; Peer

& Taborsky 2005) as well as no fitness differences compared to parental populations (Luna & Hawkins 2004; Sheffer et al. 1999; Smoker et al. 2004; Wang et al. 2006). In addition, variation in the fitness response of hybrids across generations has been demonstrated in studies where crosses were undertaken beyond the F_1, suggesting the simultaneous expression of hybrid vigour and hybrid break-down (e.g. Edmands 1999; Marr et al. 2002; Palmer & Edmands 2000).

A number of studies have examined outbreeding depression and population divergence in fish species of both conservation and commercial interest (e.g. Gharrett et al. 1999; Gilk et al. 2004; Miller et al. 2004; Smoker et al. 2004). A recent study examining the influence of outbreeding depression on disease susceptibility in inter-population hybrids of the largemouth bass (*Micropterus salmoides*) found increased mortality in F_2 offspring compared to the F_1 and parental populations (Goldberg et al. 2005). This increase in disease susceptibility was attributed to the disruption of co-adapted gene complexes in the immune system of the F_2, and provides an important empirical example of the potential for the interaction between selective agents such as infectious disease and the expression of outbreeding depression.

1.6 Can we predict patterns of local adaptation and outbreeding depression?

In a conservation context, being able to predict patterns of local adaptation and outbreeding depression would be invaluable for managers and conservation biologists considering inter-population hybridisation as a conservation action in the management of threatened species. Furthermore, understanding the factors that relate to the expression of outbreeding depression can provide important insights into the process of speciation. Knowing the central role of population divergence in the expression of outbreeding depression, can various measures or surrogates of population divergence predict outbreeding depression? There are three primary distance measures that can be used as surrogates or measure of population divergence to predict the fitness effects of inter-population hybridisation; (i) geographic distance, (ii) environmental distance, and (iii) genetic distance between the parental populations. Genetic distance can be further separated into genetic distance based on neutral molecular genetic markers (F_{ST}) and

quantitative traits (Q_{ST}). This focus of this thesis is to examine the predictive power of geographic and environmental distance.

1.6.1 Geographic distance

Geographic distance between populations is the simplest and most accessible tool for conservation managers to select appropriate source populations for the augmentation of small or declining populations, and in such cases is used as a surrogate for population differentiation and divergence. However, due to the interaction of gene flow, selection, genetic drift and environmental heterogeneity it is often difficult to predict the relationship between geographic distance and environmental and/or genetic distance (Montalvo & Ellstrand 2001). It is expected that genetic distance based on neutral molecular markers (F_{ST}) would generally follow an isolation by distance model, and thereby correlate with geographic distance between populations. In the case of quantitative genetic variation, the combined action of selection, gene flow and drift on population differentiation means that genetic distance based on quantitative traits (Q_{ST}) may be independent of F_{ST} (McKay & Latta 2002) and not necessarily correlate with geographic distance due to non-linear spatial patterns in environmental heterogeneity. Following this, for there to be a relationship between environmental and geographic distance, spatial variation in environments must change in a linear manner, such as with clinal variation, rather than a mosaic pattern of environmental variation (Edmands 2002).

1.6.2 Environmental distance

It is preferable to use a composite measure of environmental distance including a range of ecological factors such as climatic variables, soil type and elevation (Edmands 2002), since the influence of environment in generating local selection pressures will depend on these variables acting in concert (Linhart & Grant 1996). Environmental differences between populations may be expected to influence the magnitude of outbreeding depression based on dilution of genes associated with local adaptation (Montalvo & Ellstrand 2001). In this case, the different selective pressures in each environment, in combination with the action of genetic drift, will result in adaptive genetic differentiation between populations. Consequently, the relative environmental

differences between populations may be an important predictor of outbreeding depression and may closely reflect adaptive divergence.

1.6.3 Which matrix is most informative in predicting patterns of outbreeding depression?

Very few empirical studies have examined the correlation between outbreeding depression and different divergence measures such as geographic, environmental, genetic distance. The predictive power of these various matrices will depend on the relative importance of selection, drift, dispersal patterns and gene flow as well as the spatial pattern of environmental heterogeneity in determining population differentiation (Edmands 2002). The majority of studies that have examined the level of outbreeding depression in relation to any of the potential predictive matrices have used geographic distance between parental populations (e.g. Dudash & Fenster 2000; Fenster & Galloway 2000a; Hardner 1998; Sobrevila 1988; Stacy 2001). For the few studies that have combined more than one distance matrix, empirical evidence suggests that in some cases genetic and environmental distance may have more predictive power than geographic distance (Montalvo & Ellstrand 2001), while in other cases the predictive power of geographic and/or genetic distance may vary depending on the generation of inter-population hybridisation (Edmands 1999), or the fitness trait measured (Pelabon et al. 2005).

Considering that outbreeding depression can result from the complex interaction of ecological, evolutionary and genetic processes (Dudash & Fenster 2000), it may be best predicted using a combination of different predictive matrices. Furthermore, the relative importance of each predictive matrix will depend on the genetic mechanisms underlying outbreeding depression and population divergence. This thesis will examine outbreeding depression across multiple generations and fitness traits in relation to geographic and environmental distance, and assess the predictive power of these two matrices in relation to the different potential genetic mechanisms.

1.7 Outbreeding depression and *S*-alleles: A trade off?

1.7.1 *S*-alleles and population size

For plant species, genetically controlled self-incompatibility (SI) systems function to avoid the deleterious effects of inbreeding depression by preventing self-pollination or pollination among related individuals (de Nettancourt 1977; Hiscock & Tabah 2003). SI systems function effectively in large, genetically diverse populations where negative frequency dependent selection acts to maintain high *S*-allele diversity (Wright 1939). However, a decline in population size can lead to a reduction in genetic diversity at the self-incompatibility locus (*S*-locus), and can have important demographic consequences for small populations (Barrett & Kohn 1991; Byers & Meagher 1992; Young et al. 2000b). Two primary demographic responses are predicted when there is a decrease in the number of compatible mates due to the loss of *S*-alleles in small populations. These responses are (Byers & Meagher 1992): (i) a decrease in mean seed set due to a decline in the number of available mates, and (ii) an increase in the variance in seed set due to the disproportionate contribution of individuals with rare *S*-alleles. These demographic patterns have been observed in small populations of a number of self-incompatible species (e.g. Fischer et al. 2003; Luijten et al. 2000; Morgan 1999; Widen 1993), suggesting that for many species with SI systems, the loss of *S*-alleles may be an important consideration in the management of small populations.

Despite the theoretical background (Byers & Meagher 1992; Wright 1939) and potential importance of these issues for long-term population viability (Barrett & Kohn 1991; Young et al. 2000b), only a few empirical studies have examined the relationship between reduced seed set in small populations and *S*-allele diversity. The three empirical studies that have associated *S*-allele diversity to reduced seed set in small populations of self-incompatible species include studies of *Aster furcatus* (Reinartz & Les 1994), *Liatris helleri* (Godt & Hamrick 1995) and *Hymenoxys acaulis* var. *glabra* (DeMauro 1993). Young et al. (2000b) explicitly examined the relationship between *S*-alleles, mate availability and fecundity in the endangered daisy *Rutidosis leptorrhynchoides* and found that small populations had reduced *S*-allele diversity that translated directly into both a decrease in mate availability and fecundity, and increased inter-plant variance in seed set. These studies highlight the implications for population

viability of the demographic constraints of SI in small populations. In addition, very little is known about the response of polyploid populations to a reduction in population size in relation to genetic diversity at the *S*-locus. On one hand, theory predicts that polyploids may be more resilient to the loss of genetic diversity in small populations (Bever & Felber 1992) leading to maintenance of greater *S*-allele diversity in small polyploidy populations. Yet given that polyploids have a greater number of alleles per individual, there may be a greater likelihood of matching *S*-alleles which could translate to greater mate limitation in small populations.

Negative frequency dependent selection is a form of balancing selection that acts to maintain high genetic diversity at the *S*-locus (Wright 1939) and may counteract the loss of *S*-alleles through genetic drift in small populations (Schierup et al. 2000). It is, therefore, expected that balancing selection would reduce the loss of *S*-allele diversity in small populations compared to the loss of diversity at neutral loci (Fischer et al. 2003). For *R. leptorrhynchoides*, however, allozyme and *S*-allele diversity showed a similar rate of decline with decreasing population size (Young et al. 2000b). Although the effectiveness of frequency dependent selection will depend of the type of SI, such that dominance relationships among *S*-alleles in sporophytic SI systems can reduce the efficiency of frequency dependent selection (Schierup et al. 1997, 1998). This indicates that negative frequency dependent selection may not be sufficient to counteract the loss of *S*-alleles through drift, which can lead to mate limitation and demographic constraints in small populations.

1.7.2 *S*-alleles and population differentiation

The distribution of genetic variation between populations is determined by the complex interplay of the evolutionary processes of genetic drift, mutation, selection and migration (Barrett & Kohn 1991; Yeh 2000). Genetic drift and selection are the key processes involved in the differentiation of populations, while migration and gene flow will counteract the effect of these two processes through the exchange of genetic material between populations (Ellstrand & Elam 1993). Due to the role of selection in determining the distribution of genetic variation among populations, neutral loci and loci under different forms of selection may show distinctive patterns of population differentiation (Lewontin & Krakauer 1973; Podolsky & Holtsford 1995). For the *S*-

locus, negative frequency dependent selection has two important characteristics that may reduce population differentiation at the *S*-locus. Firstly, balancing selection acts to maintain high allelic diversity in populations, and secondly, it facilitates the efficient migration of *S*-alleles between populations by favouring novel or rare alleles (Brennan et al. 2006; Castric & Vekemans 2004; Schierup et al. 2000). However, despite the action of negative frequency dependent selection, the stochastic loss of *S*-alleles in small populations may result in the incomplete sharing of *S*-alleles between populations (Glemin et al. 2005; Schierup 1998; Schierup et al. 1997). Recent empirical studies in natural populations substantiate the theoretical expectations of low differentiation among populations for genetic diversity at the *S*-locus compared to neutral loci (Brennan et al. 2006; Glemin et al. 2005).

1.7.3 Genetic rescue: Is there a trade-off with outbreeding depression?

Genetic rescue in the form of increased inter-population gene flow may play an important role in ameliorating the decrease in mate availability in small populations due to reduced genetic diversity at the *S*-locus (Tallmon et al. 2004; Willi & Fischer 2005). For small populations suffering from inbreeding, the introduction of new genetic material into populations may also counteract the deleterious effects of inbreeding depression and result in an increase in mean population fitness (heterosis) (Dudash & Fenster 2000; Heschel & Paige 1995; Willi et al. 2005). An additional benefit of genetic rescue is that it may enhance genetic variation and thereby increase the long-term evolutionary potential of small, fragmented populations (Fischer & Matthies 1997; Heliyanto et al. 2006). To predict the consequences of genetic rescue for long-term population viability, however, these potential benefits to small populations need to be considered in the context of any possible negative effects of outbreeding depression. It is particularly important to examine this trade-off over multiple generations as although genetic rescue may have immediate benefits for demographic outcomes in small populations due to increased *S*-allele diversity and heterosis, outbreeding depression may not become apparent until the F_2 or later generations. Very few studies have examined the fitness consequences of genetic rescue in relation to the trade-off between introducing novel *S*-alleles, heterosis and outbreeding depression, particularly over multiple generations. For the self-incompatible species *Ranunculus reptans*, small populations were found to have a significantly higher number of incompatible crosses,

suggesting reduced *S*-allele diversity in small populations (Willi et al. 2005). For this species, genetic rescue by inter-population crosses resulted in a two-fold benefit for population fitness, firstly through increased cross-compatibility and secondly through heterosis for F_1 fitness (Willi & Fischer 2005). The impact of genetic rescue on long-term population viability will also depend on effective population size, with the relative importance of *S*-allele diversity and heterosis likely to decrease as population size increases. This suggests that population size may play in important role in determining the overall long-term outcomes of genetic rescue for mean population fitness.

1.8 Objectives and thesis outline

The primary aim of this thesis is to investigate local adaptation and outbreeding depression in *R. leptorrhynchoides* and examine the predictive power of population size and geographic and environmental distance matrices to explain patterns of local adaptation and outbreeding depression. This thesis also aims to consider these issues in the context of *S*-allele diversity in small populations and the potential benefits to fertilisation success of crossing between populations. Specifically, this thesis aims to answer the following questions:

1. Is there local adaptation in *R. leptorrhynchoides*?
2. Is there outbreeding depression when crosses are undertaken between populations? Does outbreeding depression vary across first, second and third generations?
3. What are the potential mechanisms underlying outbreeding depression?
4. What is the predictive power of population size and geographic and environmental distance matrices to explain patterns of local adaptation and outbreeding depression?
5. Does population size influence fertilisation success and *S*-allele diversity within populations? Are there benefits to fertilisation success by crossing between populations? Do these relationships vary with ploidy level?

Chapter 2 introduces the study species *Rutidosis lepttorrhynchoides* and describes the geographic distribution of remnant populations of this species. This chapter also introduces the two predictive matrices that will be used throughout this thesis to explain patterns and local adaptation and outbreeding depression. Chapter 3 examines local adaptation, while chapter 4 explores outbreeding depression. For both chapters the

power of population size and geographic and environmental distance to explain patterns of local adaptation and outbreeding depression are discussed. Discriminating the genetic basis of outbreeding depression, including the role of dilution of genes associated with local adaptation and the breakdown of co-adopted gene complexes is explored in Chapter 5. The influence of population size on genetic diversity at the self-incompatibility locus (*S*-locus) and genetic rescue are examined for diploid and tetraploid populations in Chapter 6. Chapter 7 includes a general discussion of the results of this thesis and examines the contribution and implications of this research to both the scientific understanding of the issues of local adaptation and outbreeding depression and the management of this species.

CHAPTER 2:
GEOGRAPHIC AND ENVIRONMENTAL DISTANCE

2.1 Study species: *Rutidosis leptorrhynchoides*

2.1.1 Species introduction

Rutidosis leptorrhynchoides F. Muell. (Asteraceae) is multi-stemmed herbaceous perennial with a sporophytic self-incompatibility system (Young et al. 2000a) (Figure 2.1). This species is endemic to the *Themeda triandra* dominated temperate grasslands and grassy woodland communities of South-Eastern Australia (Figure 2.2), which is a highly fragmented vegetation community that has been reduced to approximately 0.5 % of its original two million ha since the mid 1800's (Kirkpatrick et al. 1995). In parallel with the reduction in its native habitat, this species has experienced widespread declines in population size, number and geographical extent due to habitat loss and fragmentation, so that currently only 20 remnant populations remain. In addition, recent reductions in fire frequency and invasion of grassland communities by exotic weeds have contributed to the decline in the number and size of populations (Scarlett & Parsons 1990). Consequently, this species is listed as Endangered under both the NSW Threatened Species Conservation Act (1995) and the federal Environment Protection and Biodiversity Conservation Act (1999).

Rutidosis leptorrhynchoides is a cytologically complex species with two well described chromosome races (Murray & Young 2001). The 15 remnant diploid populations ($2n$ = 22) are distributed through South-East New South Wales, the Australian Capital Territory and central Victoria, while 5 remnant autoteteraploid populations ($2n$ = 44) (Brown & Young 2000; Murray & Young 2001) are located in central and western Victoria (Figure 2.3). Diploid and tetraploid plants are generally morphologically indistinguishable in both the field and glasshouse, however tetraploids have fewer florets per capitulum and larger pollen grains (Young et al. 2000a) as well as larger stomates and a lower stomatal density (M. Pickup unpublished data).

Figure 2.1 (a) A mature individual of *Rutidosis leptorrhynchoides*, (b) an inflorescence (flowerhead)

Photographer: (a) Melinda Pickup, (b) Michele Dudash

Figure 2.2 Grassland habitat of *Rutidosis leptorrhynchoides*

Photographer: Melinda Pickup

Rutidosis leptorrhynchoides grows to 20 – 40 cm in height and flowering is protracted, lasting form November to February, with each plant producing many inflorescences (flowerheads). This species is insect pollinated, although the specific insect vectors are unknown. Seed dispersal distances are commonly less than 0.5 m (Morgan 1995a) which contributes to high spatial genetic structure (Wells & Young 2002). *Rutidosis. leptorrhynchoides* 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). Individual plants have an unknown longevity but are thought to live for 10-15 years (Morgan 1999; Scarlett & Parsons 1990).

Almost half (40 %) of the 20 remnant populations of *R. leptorrhynchoides* (15 diploid and 5 tetraploid) contain < 350 reproductive individuals (Table 2.1), which has critical implications for *S*-allele diversity and mate availability in these small populations (Young et al. 2000b). Given its high conservation status at a state and federal level and the potential for reduced *S*-allele diversity in small populations, the translocation of individuals from large populations to augment small populations to restore *S*-allele diversity would be an important potential management action for this species. Therefore understanding the possible risks of outbreeding depression is vital to evaluating potential management actions and ensuring that the translocation of individuals between populations has no detrimental effects on population viability.

2.2 Geographic distance matrix and experimental design

2.2.1 Geographic distribution of populations

The current distribution of populations of *R. leptorrhynchoides* is divided into two broad geographic zones; a northern zone (< 35°30′ S, > 148°30′ E) around the Australian Capital Territory and New South Wales, and a southern zone (> 37° S, < 145°30′ E) in Victoria around Melbourne and extending into Central-Western Victoria (Figure 2.3). In the northern zone, most populations are located within 15 km around the urbanised centres of Canberra and Queanbeyan (Figure 2.4), with an additional population located near Captains Flat and another east of Goulburn approx. 80 km North-East of Canberra (Figure 2.3). In the southern zone, two populations are located

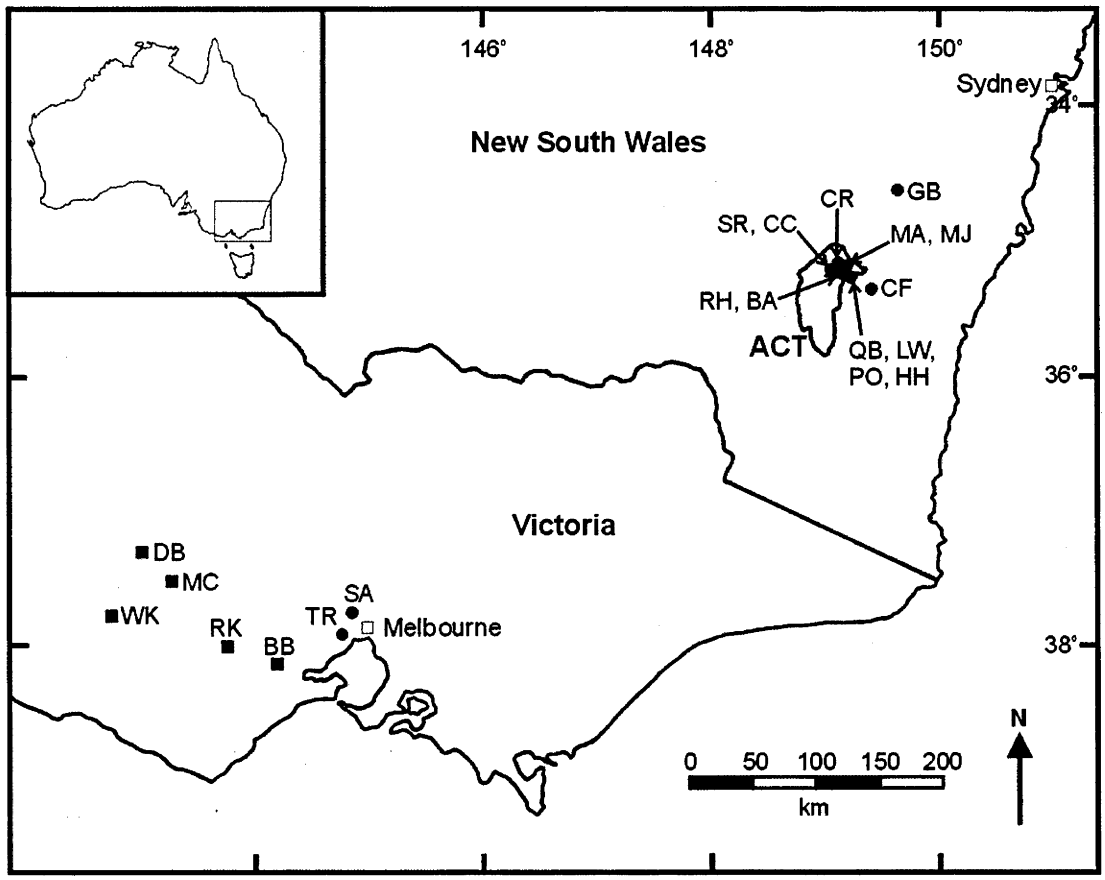

Figure 2.3 The geographic distribution of remnant populations of *Rutidosis leptorrhynchoides* across South-Eastern Australia.

Diploid populations ($2n$ = 22) (●), Tetraploid populations ($2n$ = 44) (■)

in the outer suburbs of Melbourne, while the remaining 5 populations extend into the volcanic plains of western Victoria (Figure 2.3).

2.2.2 Experimental design

2.2.2.1 Experimental rationale

As discussed in chapter 1, examining the fitness consequences of translocation first requires an assessment of the fitness of foreign (Source) plants compared to the local (Target) population (local adaptation experiment; Chapter 3). An examination of the fitness of progeny from matings between local and foreign plants (inter-population hybrids) over multiple generations is then required to assess the potential effects of translocation for long-term population viability (outbreeding depression experiment;

Chapters 4 and 5). There are a number of potential experimental designs to examine both local adaptation and outbreeding depression including; (i) a focal population design: which involves transplanting into and crossing the focal population with populations at various geographic distances from the focal population (e.g. Fenster & Galloway 2000b). (ii) Sets of populations: where populations in each set are reciprocally transplanted and/or crossed to produce hybrid progeny (e.g. Galloway & Etterson 2005). (iii) Full (or partial) diallel design: where every population is reciprocally transplanted and/or crossed with every other population (or sub-set for partial design, e.g. Paland & Schmid (2003)). (iv) Single (or multiple) population comparisons: in which a single population is transplanted into and/or crossed with one other population (e.g. Fischer & Matthies 1997; Keller et al. 2000). (v) Population pairs design: which is extended from design (iv) and involves transplanting and crossing using multiple pairs of populations over a range of spatial scales.

The merits of each particular design discussed above will depend on both the specific hypotheses being tested and logistical constraints. For example, although the full diallel design using numerous populations would provide maximum information on a species response to inter-population hybridisation, it is logistically impossible for broad scale multi-generational studies. Furthermore, generalising a species response to inter-population hybridisation is difficult using focal populations or a limited number of comparisons because population size, history and environmental factors may produce specific levels of differentiation and/or genetic architecture in a population that result in atypical patterns of hybrid fitness. The aim of this thesis in relation to examining the fitness effects of translocation and inter-population hybridisation was to design an experimental study where the results could be generalised to the species as a whole, and in which geographic and environmental distance (as surrogates of population divergence) could be used to explain patterns of variation in hybrid fitness. Therefore, using a multiple population pairs design (outlined in section 2.2.2.2), with pairs spanning a range of spatial scales, was chosen as this design enabled; (i) the inclusion of all remnant populations of this species, (ii) an examination of overall patterns of hybrid progeny fitness across many populations, and (iii) the use of predictive matrices such as geographic and environmental distance to explain patterns of local adaptation and outbreeding depression.

Table 2.1 Population size, geographic location and cytological variation in the 20 remnant populations of *Rutidosis leptorrhynchoides*.

[1]population census size based on number of reproductive adults
D = Diploid, T = Tetraploid.

Population (population code)	Geographic location	Ploidy level	Population size[1]
Wickcliffe (WK)	south	T	28
Barton (BA)	north	D	81
Campbell Park (CP)	north	D	118
St Albans (SA)	south	D	137
Captains Flat (CF)	north	D	210
Capital Circle (CC)	north	D	220
HMAS Harman (HH)	north	D	300
Bannockburn (BB)	south	T	340
Middle Creek (MC)	south	T	610
Truganina (TR)	south	D	626
Letchworth (LW)	north	D	1171
Dobies Bridge (DB)	south	T	2616
Red Hill (RH)	north	D	3489
Crace Reserve (CR)	north	D	4000
Rokewood (RK)	south	T	5149
Poplars (PO)	north	D	8171
Queanbeyan (QB)	north	D	10000
Majura Firing Range (MJ)	north	D	27626
Stirling Ridge (SR)	north	D	69600
Goulburn (GB)	north	D	95200

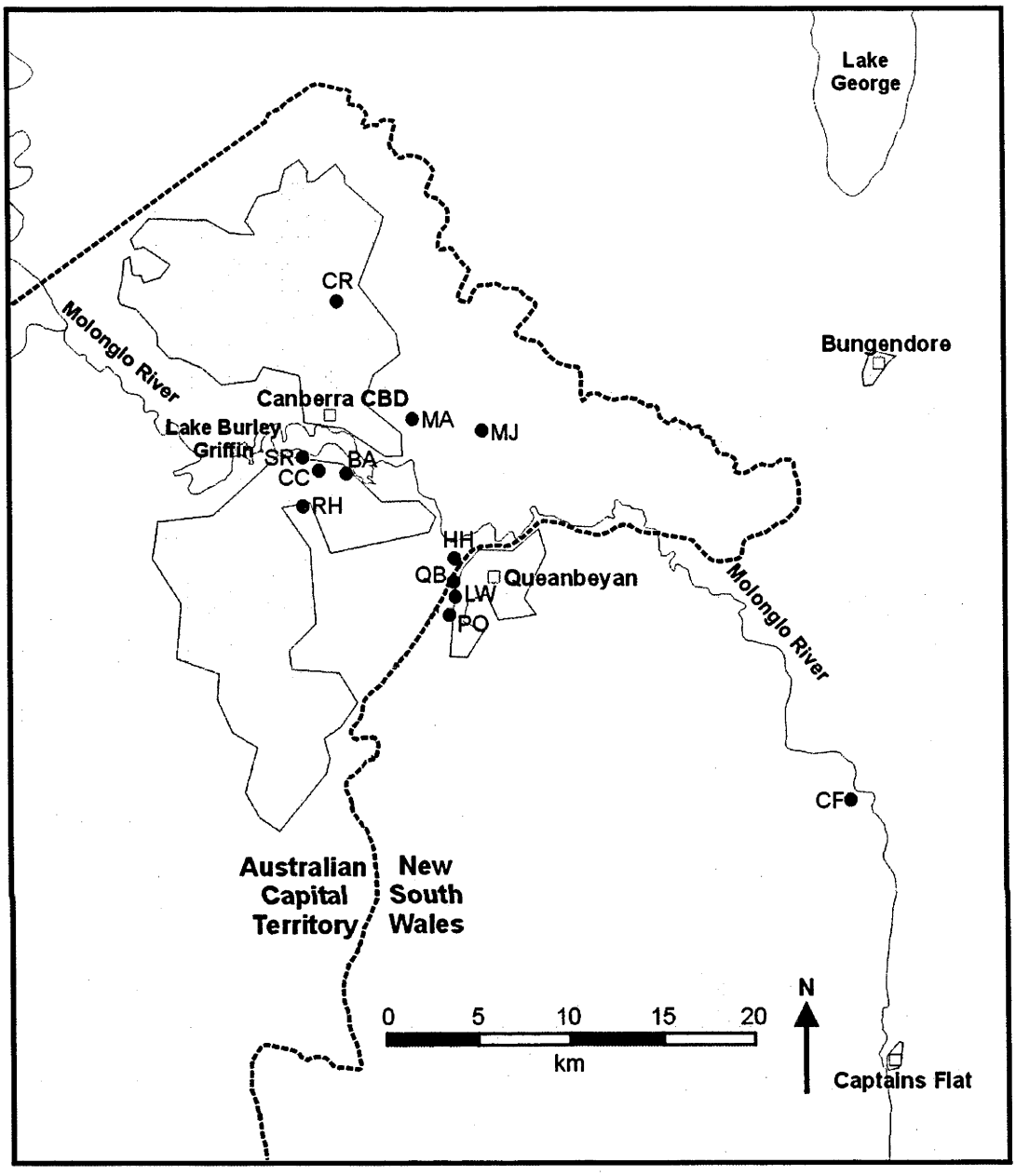

Figure 2.4 The distribution of populations of *Rutidosis leptorrhynchoides* around Canberra and Queanbeyan in South-Eastern Australia.

Each population is denoted by (•) and population code (see Table 2.2)
Grey shaded areas represent urbanised areas. ▪▪▪▪▪▪State/Territory border

2.2.2.2 Population pairs

Considering the known cytological variation in *R. leptorrhynchoides* (Murray & Young 2001) only diploid populations were considered for inclusion in the local adaptation and outbreeding depression experiments. A complete matrix of all diploid populations yielded 105 potential population pairs. The linear distances in kilometres between members of each pair were used to generate the geographic distance matrix for all populations. From this, 18 population pairs were chosen to span the geographic distribution of the species and to ensure that population pairs represented an even spread across a range of spatial scales from < 1 km to 600 km. In each pair, populations were assigned as either the Target (home) population or the Source (foreign) population. Due to the limited number of remaining *R. leptorrhynchoides* 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 minimise the number of times a population was used and to ensure the even distribution of populations as Targets and Sources. The local adaptation and outbreeding depression experiments were initially planned as field-based experiments and so the availability of scientific licences and logistical constraints associated with undertaking field-based growth trials was also taken into consideration when assigning Target and Source populations in each pair. These population pairs are outlined in Table 2.2. For the outbreeding depression experiment, a sub-set of 12 population pairs were chosen according to the above criteria.

2.3 Environmental distance matrix

Soil, climate and elevation were used to characterise the environment at each site and generate a composite measure of environmental distance between population pairs. Using a composite measure of environmental distance to quantify the differences in environment between populations is likely to provide the most appropriate surrogate for adaptive divergence, given that a number of environmental variables may act in concert to generate local selection pressures in each population (Edmands 2002; Linhart & Grant 1996). Climatic information on each site was obtained from the climate modelling program BIOCLIM 3.14 (and the Bureau of Meteorology (BOM)), elevation from GPS

Table 2.2 Geographic distance between population pairs chosen for the local adaptation and outbreeding depression experiments.

*12 population pairs used in the outbreeding depression experiment.
#For the first local adaptation experiment Truganina (TR) – St Albans (SA) (11.55 km) was used instead of Letchworth (LW) – Stirling Ridge (SR) and in population pair 15, Truganina (TR) was the Target and Stirling Ridge (SR) the Source population.

Population pair number	Target (home) population	Population code	Source (foreign population)	Population code	Geographic distance between populations (km)
1*	Letchworth	LW	Queanbeyan	QB	0.69
2*	Stirling Ridge	SR	Capital Circle	CC	1.59
3*	Campbell Park	CP	Barton	BA	3.96
4*	HMAS Harman	HH	Campbell Park	CP	7.98
5	Queanbeyan	QB	Red Hill	RH	9.56
6	Queanbeyan	QB	Stirling Ridge	SR	10.82
7	Letchworth	LW	Stirling Ridge	SR	11.4
8#	Truganina	TR	St Albans	SA	11.55
9*	Crace Reserve	CR	Letchworth	LW	15.24
10*	Majura Firing Range	MJ	Captains Flat	CF	27.81
11*	Red Hill	RH	Captains Flat	CF	34.81
12	Crace Reserve	CR	Captains Flat	CF	37.5
13*	Majura Firing Range	MJ	Goulburn	GB	71.89
14*	Goulburn	GB	Poplars	PO	78.89
15	Goulburn	GB	Capital Circle	CC	79.93
16*#	Stirling Ridge	SR	Truganina	TR	506.2
17*	Captains Flat	CF	St Albans	SA	516.01
18	Goulburn	GB	St Albans	SA	575.13
19*	Goulburn	GB	Truganina	TR	586.17

readings taken at each site and edaphic variables from soil composition and chemical soil analysis.

2.3.1 Elevation and bioclimatic variables

BIOCLIM 3.14 was used to generate 27 bioclimatic variables for each population location (listed in Appendix 2.1). For each location, climatic data obtained from BIOCLIM was compared to climatic data from the closest meteorological station from the BOM. There was close agreement between the BIOCLIM and BOM data for all sites except Goulburn (GB). At this site, BIOCLIM was found to overestimate temperature data when compared to long-term temperature records for two meteorological stations 2 – 15 km from the GB site. Therefore, for this site, temperature values from the BOM were used to calculate the following variables for use in

subsequent analysis; Annual Mean Temperature (AnMT), Mean Diurnal Range (MDR), Maximum Temperature of Warmest Period (MaxTWaP), Minimum Temperature of Coldest Period (MinTCoP), Mean Temperature of Wettest Quarter (MeTWetQ), Mean Temperature of Driest Quarter (MeTDrQ), Mean Temperature of Warmest Quarter (MeTWaQ), and Mean Temperature of Coldest Quarter (MeTCoQ). These variables were calculated to be directly comparable to those generated through BIOCLIM.

A correlation matrix was constructed for all 27 bioclimatic variables using Genstat (9th Ed.). Variables with highly significant correlations ($P < 0.001$, using a two-sided test of correlations different from zero; Genstat 9th Ed.) were removed from the data set. The five least correlated variables ($0.1 < r < 0.6$) selected for use in subsequent analysis were; Elevation (Elev), Highest Period of Radiation (HiPerRad), Mean Temperature of Wettest Quarter (MeTWetQ), Precipitation of the Driest Quarter (PrecipDQ) and Radiation of the Driest Quarter (RadDriQ) (see Table 2.3).

2.3.2 Soil variables

In each population, soil samples were collected using five equidistant sampling points along a transect in each population. Due to differences in the size and geographical area of each population, this sampling technique ensured that the five soil samples were representative of the edaphic composition of the site. After collection, these sub-samples were pooled and the bulked site sample sent to the Analytical Services Laboratory at CSIRO Land and Water, South Australia for chemical and soil particle analysis.

A correlation matrix was constructed for the 16 soil variables using Genstat (9th Ed.) As for the bioclimatic variables, soil variables with highly significant correlations ($P < 0.001$, using a two-sided test of correlations different from zero) were removed from the data set. The seven least correlated soil variables ($0.01 < r < 0.7$; with the majority $r < 0.5$) selected for subsequent analysis were; Clay (CL), Coarse Sand (CS), Copper (Cu), Manganese (Mn), Electrical Conductivity (EC), Ammonium-Nitrogen (NH_4-N) and Nitrate-Nitrogen (NO_3-N) (see Table 2.3).

Table 2.3 Bioclimatic and soil variables used in the PCA to quantify environmental differentiation between populations.

Environmental variables	Variable code
Bioclimatic variables	
Elevation	Elev
Highest Period of Radiation	HiPerRad
Mean Temperature of Wettest Quarter	MeTWetQ
Precipitation of the Driest Quarter	PrecipDQ
Radiation of the Driest Quarter	RadDriQ
Soil variables	
Clay	CL
Coarse Sand	CS
Copper	Cu
Manganese	Mn
Electrical Conductivity	EC
Ammonium-Nitrogen	NH4-N
Nitrate-Nitrogen	NO3-N

Spatial variation in the composition and abundance of soil micro-organisms may also contribute to patterns of environmental differentiation and local adaptation, since soil micro-organisms are expected to play an important role in plant community dynamics (Bever 2003). Moreover, for species dependent on nitrogen-fixing bacteria, the micro-biological soil community can play a significant role in determining patterns of local adaptation (e.g. Lie et al. 1987). In this study, however, the microbiological component of the soil community was not included as an environmental component. *Rutidosis leptorrhynchoides* is not a nitrogen-fixing species and the process of steam sterilisation to reduce the viability of weed seeds in the soil-stored seed bank in soil collected for both the local adaptation and outbreeding depression experiments (see sections 3.2 and 4.2), would have reduced the number of soil micro-organisms to negligible levels. Consequently, any spatial differences in the soil micro-biological community were not incorporated into soil differences between sites since sterilisation of the soil would have standardised this component of the environment across all sites.

2.3.3 Statistical analysis

To assess the variability in climate and soils between populations, the Coefficient of Variation (CV) was calculated for bioclimatic and soil variables across all populations and within the northern distribution zone representing 13 populations. The southern zone was excluded from this analysis as it included only two populations.

Principle Components Analysis (PCA) was used to calculate three matrices of environmental distance. This included environmental distance based on; (i) five bioclimatic variables, (ii) seven soil variables, and (iii) a composite including the 12 bioclimatic and soil variables (see section 2.3.1 and 2.3.2). For each set of variables, after the initial PCA extraction using SAS (SAS Institute), varimax rotation was applied to maximise the variance of the factor loadings and therefore improve the interpretability of the PCA. To form each matrix a Euclidean distance was calculated from the rotated factor scores for each population based on the first; (i) two components for the bioclimatic variables which explained 80.3 % of the variance, (ii) three principle components for the soil variables (77.3 % of variance explained), and (iii) three components for the composite measure that included bioclimatic and soil variables which explained 70.1 % of the variance in the data.

A Mantel test (Mantel 1967) was then used to examine associations between the difference distance matrices using Genstat (9th Ed.). In particular I was interested in assessing the correlations between geographic distance and environmental distance based on bioclimatic variables, soil variables and the composite of both bioclimatic and soil variables, as well as between the different components of environmental distance (i.e. climatic and soil distance). A Bonferroni correction was applied to assess the significance level across a number of distance matrix comparisons (adjusted alpha level of 0.0125).

2.4 Patterns of variation in environmental components and correlations between distance measures

Across all populations the Coefficient of Variation (CV) for the climatic traits was low and ranged from 0.4 – 36.8 % (with the maximum CV for 4 of the 5 variables being 11.9 %). Moreover, within the northern climate zone (representing 13 populations) the

variability in climate was even lower, with the CV across the climatic variables ranging from 0.3 – 8.6 %. In contrast, for soil variables the CV was high across all populations and within the northern climate zone. Including all populations, the CV for soil traits ranged from 48.1 – 289.3 %, and within the northern zone was between 21.3 – 71.3 %. This indicates that the variability between populations in soil characteristics is much greater than climate, even across climate zones and suggests that within these two zones, it is the variation in soil constituents and composition that is primarily driving patterns of environmental heterogeneity.

Climate distance was significantly associated with geographic distance (Table 2.4). However, as illustrated by Figure 2.5, this relationship is primarily driven by the large climatic differences between the North (ACT/NSW) and South (Victorian) climate zones (see Figure 2.3 for North-South population distributions). Populations separated by < 100 km were very similar climatically (climate distance < 1.5; Figure 2.5), with populations < 10 km apart showing very minimal differences in climate. However, when comparing populations separated by more than 500 km (representing populations from different climate zones), the climatic differences between sites more than doubled (2.5 – 3.6; Figure 2.5). This indicates that across populations the largest differences in climate are between the North-South climate zones and that within each zone populations experience relatively small differences in climatic conditions.

In comparison, the difference in soil characteristics between sites was highly variable and not associated with geographic distance between populations (Table 2.4; Figure 2.6). In some cases, populations separated by < 10 km (e.g. LW-QB; soil distance 2.9) were more differentiated on the basis of soil characteristics than populations more than 500 km apart (e.g. GB-TR; soil distance 1.5). This suggests that soil characteristics are more variable across a range of spatial scales and follow a mosaic pattern of environmental heterogeneity. Furthermore, this difference in the pattern of variation between climate and soils across populations is demonstrated by the lack of association between climate and soil distance (see Table 2.4).

Table 2.4 Correlations among different distance matrices used to describe geographic and environmental differentiation between diploid populations of *Rutidosis leptorrhynchoides*

A Mantel test based on 1000 permutations was used to test for association between the different matrices. After Bonferroni correction the alpha level for the four tests was 0.0125.
GEOGDIST = Geographic distance; CLIMDIST = Climate distance; SOILDIST = Soil distance; ENVDIST = Composite environmental distance (climate and soil variables).

	CLIMDIST	SOILDIST	ENVDIST
GEOGDIST			
r	0.93	0.37	0.46
P	<0.001	0.087	0.028
SOILDIST			
r	0.31		
P	0.064		

The composite measure of environmental distance that included both soil and climatic variables was not associated with geographic distance (Table 2.4). This likely reflects the importance of soil characteristics in determining patterns of environmental heterogeneity between sites, and has important implications for the power of both these matrices to predict patterns of local adaptation and outbreeding depression. In this case, because geographic distance is not a good surrogate of environmental distance these two matrices may reflect different patterns of population differentiation and therefore together might have the greatest power to predict local adaptation and outbreeding depression. This lack of concordance is also evident from the unweighted pairgroup method analysis (UPGMA) clustergrams (NTSYSpc v. 2.11) of geographic (Figure 2.7 a) and environmental distance (Figure 2.7 b). Hence, the environmental distance presented in subsequent chapters to predict patterns of local adaptation and outbreeding depression is the composite environmental distance matrix based on both bioclimatic and soil variables. Furthermore, understanding the contribution of climate and soils to patterns of environmental heterogeneity was central to the design and implementation of both the local adaptation and outbreeding depression growth experiments. This issue will be discussed further in this context in Chapter 3 (section 3.2.2.1).

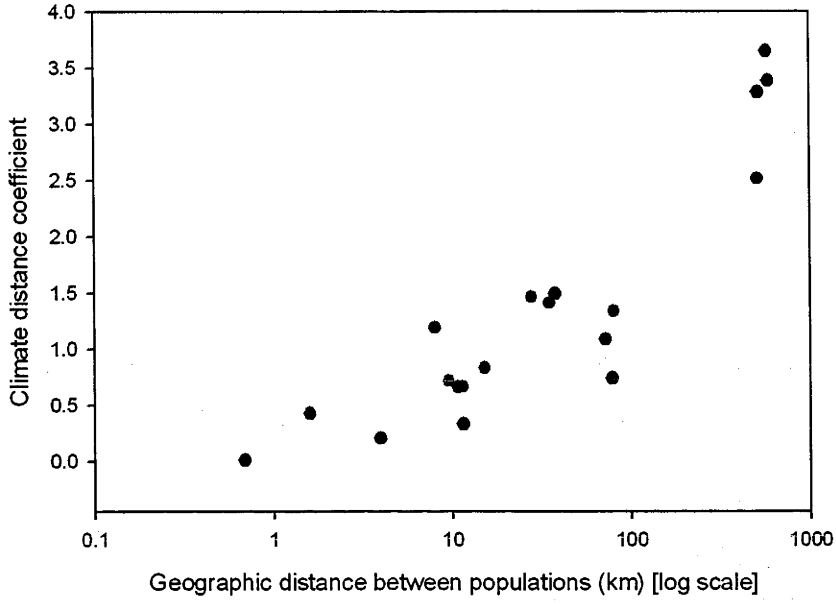

Figure 2.5 The relationship between geographic and climate distance for 19 pairs of populations of *Rutidosis leptorrhynchoides*.

Geographic distance is the linear distance (km) between populations in each pair. Climate distance is the Euclidean distance between populations based on varimax rotated factor scores from Principle Components Analysis (PCA).

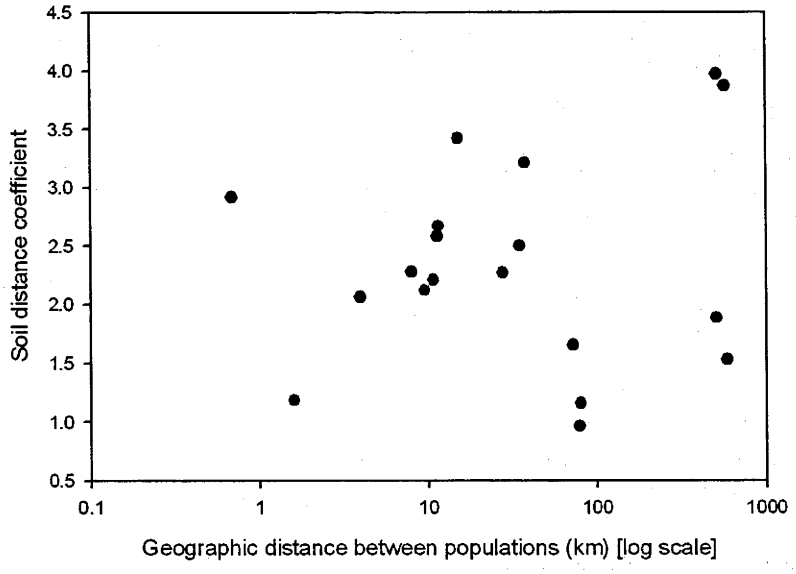

Figure 2.6 The relationship between geographic and soil distance for 19 pairs of populations of *Rutidosis leptorrhynchoides*.

Geographic distance is the linear distance (km) between populations in each pair. Soil distance is the Euclidean distance between populations based on varimax rotated factor scores from Principle Components Analysis (PCA).

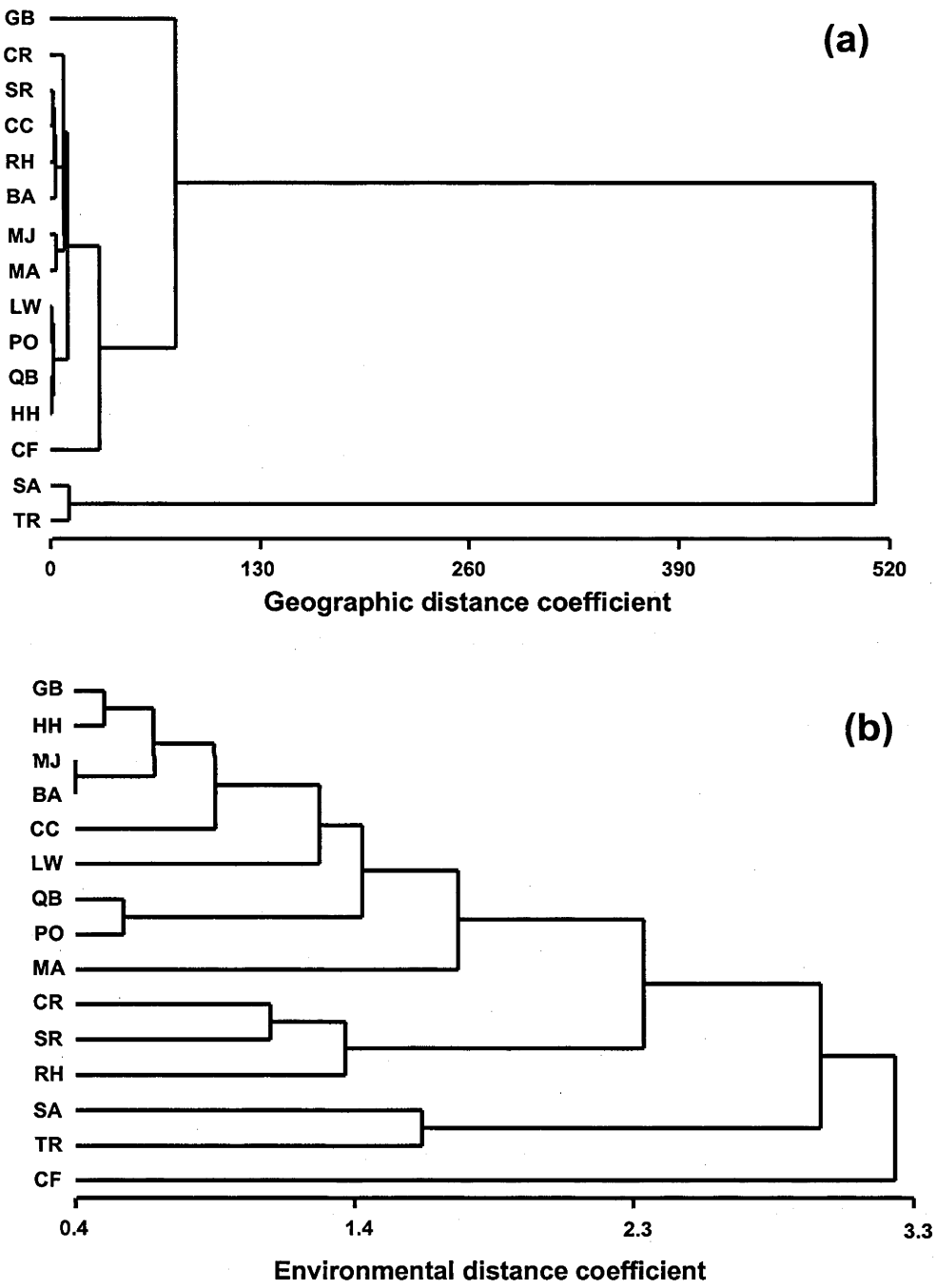

Figure 2.7 The UPGMA clustergrams of (a) geographic and (b) environmental distance among the 15 diploid populations of *Rutidosis leptorrhynchoides*.

For population codes see Table 2.1.

CHAPTER 3:
LOCAL ADAPTATION AND ADAPTIVE DIFFERENTIATION IN FRAGMENTED POPULATIONS OF *Rutidosis leptorrhynchoides*

3.1 Introduction

Local adaptation is a critical issue in the conservation and restoration of threatened plant species and is an important consideration prior to the translocation of plants between populations. This is because adaptive population differentiation, and any resulting differences in the relative performance of local and foreign genotypes, can have important implications for the success of restoration efforts and the long-term viability of restored populations (Galloway & Fenster 2000; Helenurm 1998; Montalvo & Ellstrand 2000).

Spatially divergent selection in response to specific local environmental conditions can result in adaptive differentiation through the action of both selection and genetic drift (for review see Linhart & Grant 1996). However, the relative importance of these two evolutionary processes is determined by both the strength of selection and effective population size, as drift becomes proportionally more important as population size declines (Barrett & Kohn 1991; Sherwin & Moritz 2000). In addition, gene flow between populations may counteract the development of local adaptation (Kawecki & Ebert 2004; Slatkin 1987), which highlights the importance of the interaction between selection, drift and gene flow in determining the trajectory of adaptive evolution within populations.

Although the results of individual local adaptation studies tend to be idiosyncratic and range from equivalent performance of local and foreign genotypes (e.g. Galloway & Fenster 2000; Gordon & Rice 1998; Helenurm 1998) to evidence of strong local adaptation (e.g. Kittelson & Maron 2001; Sambatti & Rice 2006), a recent meta-analysis found consistent evidence for local adaptation (Leimu & Fischer in review).

Yet despite theoretical expectations (see section 1.3.5), local adaptation was not related to plant life history or spatial and temporal habitat heterogeneity. Instead population size was found to be the key factor determining local adaptation, with local adaptation found in large, but not small populations (Leimu & Fischer in review).

Understanding the role of population size in determining patterns of adaptive population differentiation is particularly relevant to the restoration and management of threatened plant species, because small populations are the primary target for augmentation and restoration efforts. In addition, the influence of population size on local adaptation has important implications for the evolutionary trajectory of small populations, particularly in response to rapid anthropogenic environmental change (Leimu & Fischer in review). This influence of population size on local adaptation may be due to several reasons including; (i) a reduction in heritable genetic variation (Stockwell et al. 2003; Willi et al. 2007) and the efficacy of selection relative to genetic drift as population size declines (Leimu & Fischer in review; Linhart & Grant 1996), (ii) a reduction in the chance creation of beneficial mutations in small populations (Leimu & Fischer in review; Whitlock 2000), and (iii) that the homogenising effects of gene flow may be greater in small populations (Holt & Gomulkiewicz 1997; Jakobsson & Dinnetz 2005). Consequently, population size may be a key predictor of local adaptation and patterns of adaptive differentiation.

The spatial scale of local adaptation can provide important insights into the scale of adaptive evolution (Galloway & Fenster 2000). Previous studies have found examples of local adaptation across a range of spatial scales (reviewed in Linhart & Grant 1996), suggesting that it is the spatial scale of environmental heterogeneity that may drive the scale of adaptive population differentiation. In addition, a recent meta-analysis (Leimu & Fischer in review) found that although local adaptation was not significantly associated with geographic distance between populations, there was greater variation in local adaptation at small compared to large spatial scales. This indicates that at small spatial scales environmental heterogeneity can be large enough to result in local adaptation, but that this is less consistent than at large spatial scales. This is substantiated by a recent study by Bischoff et al. (2006) where, in some cases, local adaptation was found to be more prominent over small spatial scales with high

environmental differentiation compared to large spatial scales. Moreover, the relationship between selection and gene flow will change with geographic distance between populations. In this case, at smaller spatial scales with higher levels of gene flow, stronger local selection regimes are required to overcome the homogenising effects of gene flow and for local adaptation to develop (e.g. Antonovics & Bradshaw 1970; Sambatti & Rice 2006).

Given the role of environmental heterogeneity in driving patterns of local adaptation, environmental distance is expected to be a good predictor of local adaptation, as has been demonstrated empirically for the subshrub *Lotus scoparius* (Montalvo & Ellstrand 2001). In addition, relatively few studies (Becker et al. 2006a; Galloway & Fenster 2000; Montalvo & Ellstrand 2000) have explicitly examined patterns of adaptive differentiation over a range of spatial scales relevant to species distributions. Consequently, further empirical studies are required that test the expected relationship between local adaptation and geographic and environmental distance between populations.

To accurately assess the consequences of translocation for mean population fitness it is important to examine if local and foreign genotypes exhibit fitness differences in traits of greatest demographic importance. Elasticity analysis can be used to identify fitness traits at different life stages which are most significant to demographic outcomes (Ouborg & Van Treuren 1997), and enables fitness components to be interpreted in a demographic context (Crone 2001). Previous demographic work for *R. leptorrhynchoides* (Young et al. 2000b) 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. Combining this information with the results of growth experiments examining the fitness of local and foreign genotypes would enable more accurate predictions of the potential outcomes of translocation for population viability.

The aim of the research presented in this chapter is to examine patterns of local adaptation in relation to geographic and environmental distance and population size by assessing the fitness of local and foreign plants in 18 pairs of populations over two

experiments. I also aim to investigate patterns of local adaptation considering traits identified through elasticity analysis as having greatest demographic importance. Specifically, this chapter addresses the following six questions:

1. Is there evidence of local adaptation in populations of *Rutidosis leptorrhynchoides*?
2. Is the expression of local adaptation environmentally dependant? (Is there variation in the expression of local adaptation between the two experiments?)
3. Is there local adaptation for traits with high elasticity values?
4. Can spatial scale and environmental distance between populations predict patterns of local adaptation?
5. Are patterns of local adaptation related to population size?

3.2 Materials and methods

3.2.1 Local adaptation experiment 1: Field experiment

3.2.1.1 Seed collection

For all 18 population pairs, seed from the Target and Source populations were collected from 1 – 3 open-pollinated inflorescences of 24 – 60 maternal plants in December 2002 – January 2003. Maternal plants were evenly distributed along a transect through each population. For plants where the inflorescence had matured but the seed had not yet dehisced, tulle bags were placed over the inflorescence and seed collected after 2 – 3 weeks. This was done to ensure seed was sampled from throughout the population. From the potential pool of 24 – 60 maternal plants from each population, 15 maternal plants were chosen at random and removed for each Target and Source population in each population pair. This ensured that different maternal families were used in each population pair that shared either a Target or Source population. The exceptions were the Captains Flat (CF) and St Albans (SA) populations, where due to low plant numbers, population pairs 9 (MJ-CF) and 11 (CR-CF), and population pairs 7 (TR-SA) and 16 (CF-SA), shared seed from some of the same maternal plants.

3.2.1.2 Soil collection

In March 2003, soil was collected from each Target population and steam sterilised at 60 – 65°C for 45 minutes to reduce the viability of weed seeds in the soil-stored seed bank. To compensate for the loss of soil structure and permeability associated with disturbance, collected sterilised soil was mixed in a ratio of 80:20 with river sand. Soil was placed in biodegradable 6 cm 'jiffy' pots (0.1 L capacity) and arranged in blocks of 48 pots.

3.2.1.3 Experimental planting

From each open-pollinated family in each population pair, 12 seed were selected and weighed in bulk on a 4 dpl gram balance. Seeds were then cold treated in a refrigerator set at approx. 5°C for 72 hours. After cold treatment, three seeds from each mother were planted in each of four pots containing soil from the Target site. For each population pair, 60 pots (15 mothers) were planted for both the Target and Source populations, giving a total of 120 pots per population pair, and 2160 plants for the entire experiment (18 population pairs). *Rutidosis leptorrhynchoides* is an autumn germinating species with a transient seed bank, so that recruitment is dependent on seed production in the preceding season (Morgan 1995a). In the field, seedlings germinate and grow to the 2 – 6 leaf stage in autumn and then undergo minimal growth until spring. To ensure that the experiment was biologically realistic, seed collected from the previous summer was planted from the 22 – 28 April 2003. The four pots containing seed from each maternal plant were placed adjacently in blocks of 48 pots. However, to minimise any micro-scale environmental differences between blocks, the position of each block in the outdoor enclosure was randomised and then re-randomised every week for the 6 months prior to transplantation into the field in September 2003.

Germination and survivorship were scored weekly for the first 3 months. At 12 weeks, for pots where multiple seed had germinated, seedlings were culled to leave 1 seedling per pot. This plant was randomly chosen by selecting the seedling closest to the geometric centre of the pot. Where multiple seedlings had germinated in one pot, but no seedlings from a corresponding seed origin (local or foreign) and maternal line had germinated and/or survived to 3 months in another pot, seedlings were transplanted between pots to maintain sample size and maximise maternal replication before

transplantation. Before transplantation at 6 months, seedling survivorship was assessed and a number of growth variables were measured to provide a baseline measure of plant growth including:

1. Survivorship (SURV): For each seed origin (local or foreign), individuals were pooled for each maternal line and SURV calculated on a family basis.
2. Rosette height (measured as the perpendicular height from the soil surface to the maximum point on the rosette) (RosHT).
3. Rosette width (measured as the maximum diameter of the rosette) (RosW).
4. Number of leaves (NoLVS).
5. Length of the longest leaf (LLLeaf).
6. Width of the longest leaf (WLLeaf).

Two additional fitness variables were also generated using the above measurements;

7. Leaf size (LFS): LLLeaf *x* WLLeaf (mm^2)
8. Index of Plant Size (INDPS): NoLVS *x* LFS

3.2.1.4 Field site selection and preparation

The 18 population pairs were distributed across 11 field sites that correspond to the Target population in each pair. Sites with multiple population pairs include Queanbeyan (QB-RH; QB-SR), Truganina (TR-SA; TR-SR), Crace Reserve (CR-LW; CR-CF), Majura Firing Range (MJ-CF; MJ-GB) and Goulburn (GB-CC; GB-PO; GB-TR; GB-SA). Within the Target population field site, each block was located greater than 30 m but no more than 200 m from the existing population at the site. These distances were chosen to minimise the likelihood of contamination of the local population given that *R. leptorrhynchoides* has a transient seed bank (Morgan 1997) and limited seed dispersal, with most seed dispersing within 1 m of the maternal plant (Morgan 1995b; Wells & Young 2002). In addition, the location of each block was chosen to incorporate maximum environmental heterogeneity at each site.

For all population pairs, two 3 *x* 5 m areas were selected to represent the two blocks at each site. In each block the above ground biomass was removed using a brush cutter to ensure that competition for light was not confounding seedling establishment and survival and to standardise each experimental site. Each 3 *x* 5 m block was divided into 0.5 *x* 0.5 m grids since *R. leptorrhynchoides* is a gap-sensitive species that requires a

canopy gap of at least 0.3 m for successful recruitment and survival (Morgan 1997). For all pairs, 60 seedlings were planted in each of the two blocks, which included 30 seedlings from the Target population and 30 seedlings from the Source population. Two seedlings from the 15 maternal plants from the Target and Source populations were represented in each block and the position of each seedling within the two blocks was completely randomised. Across the 11 sites planting of this experiment occurred from the 15 – 30 September 2003.

A 20 cm diameter posthole digger was used to dig a cavity approximately 20 – 25 cm deep in each grid. This hole was then back-filled with the loosened soil and the jiffy pot planted in the centre of the grid. This procedure loosened the soil, improved drainage and ensured that there was a loosened zone of soil to enable root expansion for the planted seedling. A metal tag containing an engraved sequential number identified the seedling in each grid. Fences were erected around each block in sites where there was the risk of grazing by kangaroos or cattle, or the potential for pedestrian traffic. At each site plants were watered after transplantation, then subsequently at 2 and 4 weeks. In all population pairs, seedling survivorship was monitored every 4 weeks for the first 3 months.

3.2.2 Local adaptation experiment 2: Common climate and variables soils

3.2.2.1 Experimental design

Due to a combination of prevailing drought (and vandalism at three sites), survivorship of seedlings in the field transplant experiment was reduced to zero for all population pairs at 3 months. Consequently, to assess local adaptation in *R. leptorrhynchoides*, a more controlled environment was required that would still provide a biologically realistic assessment of local adaptation. Local adaptation is likely to be expressed only when plants are exposed to local environmental conditions. Field experiments provide the ideal experimental framework to assess local adaptation as the field environment includes edaphic conditions, climate, biological interactions and vegetation composition. However, defining the components of the environment that differentiate sites can provide important information on the environmental variables that drive patterns of local adaptation. Understanding these environmental components ensures

that the variables likely to be of primary importance to local adaptation are incorporated into the experimental design.

As discussed in section 2.3, multivariate analysis of bioclimatic and edaphic variables across all sites highlighted two distinct climate zones that align with the North-South geographical divide of populations of *R. leptorrhynchoides*. However, within each climate zone soil characteristics were found to differentiate sites (see section 2.4), indicating that within a climate zone it is the edaphic variability between sites that is potentially driving patterns of environmental heterogeneity. This suggests that planting seed from the Target and Source populations into soil from the Target population and in a climate representative of the Target site would provide a biologically realistic experimental framework in which to assess local adaptation. On this basis, for each population pair in the second local adaptation experiment, soil was collected from the Target population and plants were grown in a common climate representative of the northern climate zone at CSIRO Plant Industry in Canberra, ACT. 16 of the 18 population pairs used in the first local adaptation experiment remained unchanged in the second experiment. However, for the population pair TR-SR, SR replaced TR as the Target population. In addition, LW-SR (11.40 km; see Table 2.2) was substituted for TR-SA (11.55 km). These two population pairs were altered to ensure that the common climate in the northern climate zone was representative of the Target populations.

3.2.2.2 Seed collection

For all population pairs, 1 – 3 open-pollinated inflorescences were collected from 27 – 60 maternal plants during January – February 2004 according to the methods outlined in section 3.2.1.1. From the potential pool of 27 – 60 maternal plants from each population, 15 maternal plants were chosen at random and removed for each Target and Source population in each population pair. As for the first local adaptation experiment, this meant that different maternal families were used in the pairs where a population was used more than once (as either a Target or Source). For population pair 16 (CF-SA), low seed numbers meant that only 12 maternal families were available for use in this population pair.

3.2.2.3 Soil collection

For each population pair, soil was collected from the Target population. To minimise disturbance at each site and to ensure that soil used in this experiment represented an average soil profile for each site, soil was collected from the top 30 cm of the soil profile at 5 – 6 sub-sampling sites across the population. The amount of soil required from each Target population ranged from approximately 100 – 312 L. The soil from each site was then transported to CSIRO Plant Industry in Canberra and subsequently steam sterilised at 60 – 65°C for 45 minutes to reduced the viability of weed seeds within the soil-stored seed bank. To compensate for the loss of soil structure and permeability with disturbance, the sterilised soil was mixed in a ratio of 80:20 with river sand and placed in 10 cm diameter 0.5 L capacity pots.

3.2.2.4 Experimental planting

From each open-pollinated family, 12 seed were selected and weighed in bulk on a 4 dpl gram balance. Seeds were then cold treated in a refrigerator set at approx. 5°C for 72 hours. As discussed in section 3.2.1.3, *R. leptorrhynchoides* is an autumn germinating species and so planting for this experiment was undertaken from the 10 – 17 May 2004. For each of the 15 maternal families from the Target and Source populations in the 18 population pairs, 3 seed were planted into each of 4 pots containing soil from the Target population. This gave a total of 60 pots for the Target and Source populations and a total of 120 for each population pair. Across all population pairs this gave a total of 2136 pots and 6408 planted seed.

Prior to planting of each population pair, the 15 maternal families from the Target population were randomly paired with a maternal family from the Source population. In each of the four blocks, one pot containing seed from the first Target maternal family was placed adjacent to a corresponding pot from the first maternal family from the Source population. Each pair of pots was allocated to a random row and column position on benches within each block and each pot was identified only by a consecutive number corresponding to its random position. This was done so that subsequent monitoring could be undertaken without knowledge of plant identity. The four blocks used in this experiment were distributed across an outdoor enclosure at CSIRO Plant Industry, Black Mountain laboratories in Canberra, ACT. Pairing Target and Source

plants within each block accounted for any micro-scale climatic differences within each block and therefore provided a more powerful experimental design to examine the relative difference in the performance of the Target (local) and Source (foreign) plants. For each population pair this design resulted in 60 independent comparisons of the relative performance of plants from the Target and Source populations representing 15 maternal families (except for CF-SA where 48 comparisons represented 12 families).

Within the outdoor enclosure, natural precipitation was supplemented with water from an overhead watering system every 1 – 3 days (depending on the season) to ensure pots were not desiccated. Weed seeds that survived steam sterilisation and germinated within pots were removed as required. In the 2 week period after planting, soil within the pots in the outdoor enclosure was found to be freezing due to unseasonably low autumn temperatures. To avoid these unrealistically severe conditions the 2136 pots were moved to a 'cold-frame' enclosure at CSIRO Plant Industry. In this enclosure, plants were positioned at ground level and each section surrounded by a concrete barrier (Figure 3.1 a). Blocking structure and random position were maintained within the 'cold-frame' enclosure and natural precipitation was supplemented with hand-watering every 1 – 2 days as required. After a 6 month period, plants were moved back to the outdoor enclosure (Figure 3.1 b).

3.2.2.5 Monitoring

Germination and survivorship were scored weekly for the first 3 months and these data were used to assess maximum germination (GERM) and seedling survivorship ($SURV_3$). Seedling survivorship ($SURV_3$) was calculated on a family basis so that individuals were pooled across the four blocks and survivorship assessed for each maternal line. At 3 months, for pots where multiple seed had germinated, seedlings were culled to leave one seedling per pot. This plant was randomly chosen by selecting the seedling closest to the geometric centre of the pot.

To assess the relative difference in the performance of Target (local) and Source (foreign) plants, ten different variables including survivorship, growth and reproductive characteristics were measured at 12 and 24 months. These variables included:

1. Survivorship (SURV): For each seed origin (local and foreign), individuals were pooled across the four blocks and survivorship assessed for each maternal line.

2. Plant height (HT): measured as the perpendicular height from the soil surface to the maximum point on the plant.
3. Length of the longest stem (LLST): measured as the stem length from the rosette to the base of the inflorescence.
4. Number of leaves (NoLVS): This included counting all photosynthetically active leaves that were greater than 25 % expanded in the basal rosette and on the stems. Leaves were considered photosynthetically active if less than 50 % of the leaf had senesced.
5. Length of the longest leaf (LLLeaf).
6. Width of the longest leaf (WLLeaf).
7. Number of stems (NoST).
8. Number of flowering stems (FLOWERST).
9. Number of flowerhead (NoF).
10. Number of buds (NoBD).

A number of additional fitness variables were also generated using the above measurements including;

11. Leaf size (LFS): LLLeaf *x* WLLeaf (mm^2)
12. Index of Plant Size (INDPS): NoLVS *x* LFS
13. Number of Flowerheads and Buds (NoF&B): NoINF + NoBD

At 24 months, above (AGB) and below (BGB) ground biomass samples were obtained from all surviving plants. Below ground biomass was sampled by cutting the basal rosette at ground level and then washing the soil from the root mass. Above ground samples included all biomass above ground level including the basal rosette and stems. Both above and below ground biomass samples were oven-dried at 70°C for 3 days. Due to a significant correlation between AGB and BGB for samples from the outbreeding depression experiment (see chapter 4; $r = 0.71$, $P < 0.001$), which were weighed prior to the samples for the local adaptation experiment, only above ground biomass samples were subsequently weighed for this experiment. All AGB samples were weighed on a 4 dpl gram balance.

A cumulative fitness index (CFI_{24}) was generated to quantify plant fitness across the lifecycle using Germination (GERM), the proportion of Flowering Stems at 12 months ($FLOWERST_{12}$) (as a proxy for reproductive output) and Above Ground Biomass at 24

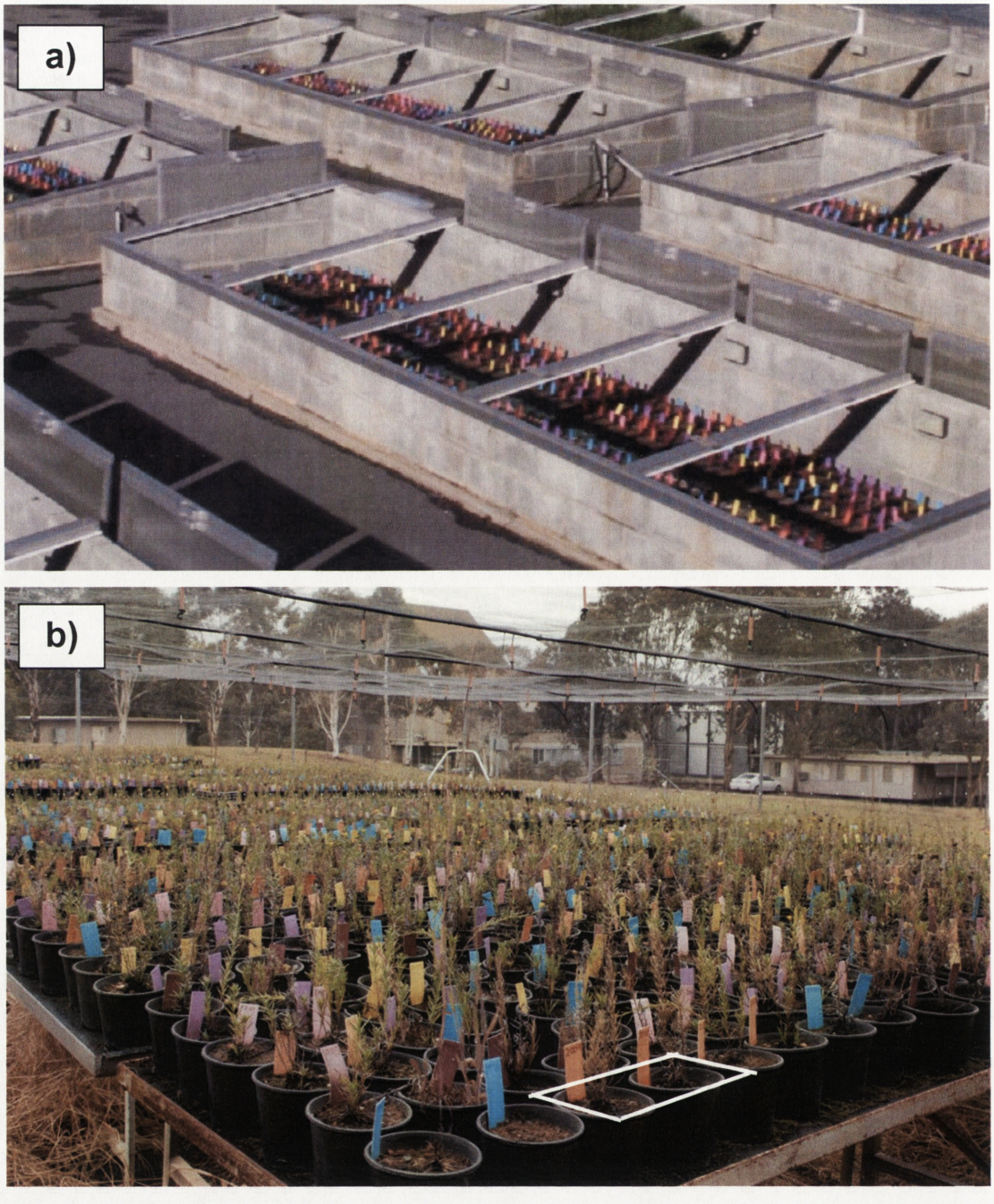

Figure 3.1 A photo of the a) cold-frame, and (b) outdoor enclosure at CSIRO Plant Industry, Canberra.

Plants for the local adaptation experiment were grown in the cold-frame enclosure for the initial six months over winter (see section 3.2.2.4), and in the outdoor enclosure for the remaining 18 months of the experiment. The white box in Figure 3.1 b represents a pair of plants (one Target and one Source plant) from a single maternal line and population pair.

months (AGB_{24}). Above ground biomass (AGB_{24}), as a measure of plant size, was used as a surrogate for reproductive adult survivorship because across all population pairs in this experiment the majority of plants that survived to 3 months ($SURV_3$) subsequently survived to reproduction ($SURV_{12}$) and to 24 months ($SURV_{24}$). For each population pair, AGB for each plant was converted to a proportion of the maximum AGB for that population pair. Therefore;

$CFI_{12} = (GERM \times FLOWERST \times AGB_{PROPMAX})$, where

GERM = Proportion germinated,

FLOWERST = Proportion of flowering stems at 12 months, and

$AGB_{PROPMAX}$ = Above ground biomass as a proportion of the maximum in each population pair.

3.2.3 Statistical analysis

All analyses were undertaken using Genstat for Windows 9th Edition (VSN International, Oxford UK). For all variables for both local adaptation experiments (and for each time period (12 and 24 months) for the second local adaptation experiment), exploratory data analysis was undertaken to assess the distribution of the data and examine the assumption of homogeneity of variances. For all analyses outlined in the following section, residual plots were assessed to check the adequacy of the models and the assumptions of normality and homogeneity of the variance. Unless stated, all statistical tests were significance tested at $\alpha = 0.05$.

Similar statistical models (i.e. GLM's and REML's; see sections 3.2.3.5, 3.2.3.6 and 3.2.3.7) were used to analyze the germination, survivorship and growth data for both local adaptation experiments (with data on seedling growth (at 6 months) for experiment 1 and adult growth and reproduction at 12 and 24 months for experiment 2). Therefore, for brevity, the following statistical methodology is presented on the basis of each analysis type and may contain (where applicable) information on the statistical models used to analyse both local adaptation experiments.

3.2.3.1 Preliminary analysis

For the first local adaptation experiment, a paired t-test was used to assess if transplantation at 12 weeks (see section 3.2.1.3) had a significant effect on subsequent

plant growth at 6 months (which corresponds the growth measurements taken prior to planting of the field experiment). For this analysis, each transplanted seedling (n = 21) was paired with a non-transplanted seedling from the same maternal family and seed origin (local or foreign) and a one-sided paired t-test used to test the null hypothesis that the growth of non-transplanted seedlings was not greater than transplanted seedlings. For all 5 variables (NoLVS, RosHT, LFS, INDPS and RosDiam) there was no significant difference ($P > 0.05$) (data not shown) between the growth of transplanted and non-transplanted seedlings. Therefore, growth data for the 21 transplanted individuals was included in the final analyses of the first local adaptation experiment.

3.2.3.2 Covariates

Due to the potential influence of seed size on germination and seedling fitness, seed weight was fitted as a covariate in the GLM models for germination and seedling survival for both local adaptation experiments and for the REML models for seedling growth in the first local adaptation experiment. The relationship between seed size and seedling fitness is well established empirically (e.g. Gomez 2004; Gross 1984; Simons & Johnston 2000; Stanton 1984; Stock et al. 1990), and although it is predicted that the influence of maternal seed reserves on plant fitness should decrease over time due to the increasing importance of other factors such as genotype and environment, a number of studies have found a relationship between seed size and adult fitness and reproduction (Simons & Johnston 2000; Stanton 1984). Therefore, for the second local adaptation experiment, seed weight was also included as a covariate in subsequent growth analysis at 12 and 24 months to ensure that the potential influence of seed size on plant fitness at the adult life-stage was incorporated in the models. However, for the overall analysis and seven individual population pairs (SR-CC, MA-BA, CR-LW, MJ-CF, RH-CF, CR-CF AND MJ-GB) in the first local adaptation experiment, and for six population pairs (QB-RH, QB-SR, LW-SR, CR-LW, RH-CF and GB-TR) in the second local adaptation experiment, there was a significant relationship between seed weight and seed origin (local or foreign) ($P < 0.05$; see Table 3.1, Appendix 3.1 and Appendix 3.2). An important assumption of Analysis of Covariance (ANCOVA) is independence between the factor and covariate. Therefore, due to collinearity between seed weight and seed origin, seed weight was not fitted as a covariate for analysis of the seven significant population pairs and for the overall analysis for the first local adaptation experiment, or

for the six significant pairs in the second local adaptation experiment. Seed weight was included as a covariate, however, for the remaining 11 population pairs in the first local adaptation experiment and for the overall analysis and 12 non-significant pairs in the second local adaptation experiment.

3.2.3.3 Repeated measures

For the second local adaptation experiment, a Split-Plot Repeated Measures ANOVA was used to examine if the effect of seed Origin (local or foreign) on plant fitness varied between the two seasons as measured at 12 and 24 months (i.e. if there is a significant interaction between Origin and Time over these two time periods). Number of leaves (NoLVS), Leaf size (LFS), Height (HT), Index of plant size (INDPS), Number of stems (NoST) and Number of flowerheads and buds (NoF&B) were the growth variables included in the repeated measures analysis. In this model seed weight was fitted as a covariate for the overall analysis across all population pairs and for the analysis for each population pair (except for population pairs QB-RH, QB-SR, LW-SR, CR-LW, RH-CF and GB-TR; see section 3.2.3.2). Origin, Time and Origin *x* Time were fitted as main effects in the fixed model, while Maternal Line *x* Block, Plant Number and Date were fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model.

3.2.3.4 The interaction between local adaptation and environment

Germination (GERM) and seedling survivorship ($SURV_3$) data for the 16 population pairs common to both experiments (see Table 2.2) were used to assess if the expression of local adaptation varied between different seasons, as these two traits were directly comparable between the two experiments. The effect of Origin (local or foreign), Experiment (Local adaptation experiment 1 and 2) and the interaction between Origin and Experiment (Origin *x* Experiment) was analysed using a Generalised Linear Model (GLM) (logistic regression) with a binomial distribution and a logit link function. Due to differences in the blocking structure between the first and second local adaptation experiments, the effect of block was excluded from this analysis. For the analysis for each individual population pair, Maternal Line (nested within Experiment) and Origin *x* Experiment (which expands to Origin + Experiment + Origin *x* Experiment) were fitted sequentially into the model. For the analysis across all population pairs, Maternal Line

and Experiment (Experiment/Maternal Line) were nested within population pair. Although both Origin and Experiment were included in the model individually, the focus of this analysis was to examine if there was a significant interaction between Origin and Experiment across all population pairs (Overall) and for each individual population pair comparison.

3.2.3.5 Generalised Linear Models (GLM) – Logistic Regression

The effect of Origin on the binary response variables including Germination (GERM) and Survivorship (SURV) (for both local adaptation experiments) and the proportion of flowering stems ($FLOWERST_{12}$) (in the second experiment) were analysed using a Generalised Linear Model (GLM) (Logistic Regression) with a binomial distribution and logit link function. For the first local adaptation experiment Seed Weight (as a covariate; except for in the overall analysis and for significant population pairs, see section 3.2.3.2), Maternal Line and Origin were fitted sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair (i.e. Population pair/Maternal line). For the second experiment Seed Weight (as a covariate; except for significant population pairs, see section 3.2.3.2), Maternal Line *x* Block and Origin were fitted sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair (i.e. (Population pair/Maternal line) *x* Block). For both experiments, Accumulated Analysis of Deviance was used to assess the significance of the overall model and each term individually. The comparison between Origins (Target (local) and Source (foreign)) was assessed as significant if the difference in the predicted means was greater than the Least Significant Difference (LSD) (at $\alpha = 0.05$) and by examining the *P* value for the individual linear comparison between the reference level (Target) and Source Origin.

3.2.3.6 Generalised Linear Models (GLM) – General Model

For the second local adaptation experiment, differences between the two Origins in Cumulative Fitness Index (CFI_{24}) were assessed using a Generalised Linear Model (GLM) with a Poisson distribution and logarithm link function. Given that the CFI_{24} was not normally distributed, the relationship between the mean and variance (and m^2 and variance) was used to identify the appropriate distribution and choice of link function for this analysis. For this model Seed Weight (as a covariate; except for

population pairs QB-RH, QB-SR, LW-SR, CR-LW, RH-CF and GB-TR), Maternal Line *x* Block and Origin were fitted sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair (i.e. (Population pair/Maternal line) *x* Block). Accumulated Analysis of Deviance was used to assess the significance of the overall model and each term individually. The comparison between Origins (Target (local) and Source (Foreign)) was assessed as significant if the difference in the predicted means was greater than the Least Significant Difference (LSD) (at $\alpha = 0.05$) and by comparing the *P* value for the individual linear comparison between the reference level (Target) and Source Origin.

3.2.3.7 Restricted Maximum Likelihood (REML) Linear Mixed Model

For the normally distributed variables in the first (i.e. seedling traits: NoLVS, RosHT, LFS and INDPS) and second (i.e. adult growth and reproductive traits; $NoLVS_{12,24}$, $HT_{12,24}$, $INDPS_{12,24}$, $LFS_{12,24}$, $NoST_{12,24}$, $NoF\&B_{12,24}$ and AGB_{24}) local adaptation experiments REML Linear Mixed Models were used to analyse differences between the two Origins across all population pairs (Overall) and for each population pair independently. Although this fitness trial was designed as a balanced experiment, mortality of individuals during the course of the experiment meant that the final data set was unbalanced. Consequently, a REML analysis of Linear Mixed Models was most appropriate to examine the effect of Origin on plant fitness. The Target (local) population was chosen as the reference level for all analyses. For the first local adaptation experiment seed weight was fitted as a covariate (except for overall analysis and seven population pairs, see section 3.2.3.2) and Origin fitted as the main effect in the fixed model, while Maternal Line was fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model. For the second local adaptation experiment, seed weight was fitted as a covariate (except for six population pairs, see section 3.2.3.2) and Origin fitted as the main effect in the fixed model, while Maternal Line *x* Block (which includes Maternal Line + Block + Maternal Line *x* Block) was fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model. Significant differences between the two Origins (local and foreign) were assessed using the Least Significant Difference (LSD), which is defined (at $\alpha = 0.05$) as twice the standard error of the difference of the means for each comparison. Therefore,

when the difference between the predicted means was greater than the LSD the comparison was considered significant. However, for all Origin comparisons, LSDs were only reported as significant if the *P* value for the global significance test was < 0.05.

A summary of the results of the REML analysis examining the effect of Origin on plant fitness for both experiments is presented for all fitness traits in Appendix 3.1 (first local adaptation experiment) and Appendix 3.2 (second local adaptation experiment). However, due to significant correlations between most variables at each life stage (data not shown), more detailed results and analysis are only presented for a sub-set of these growth variables for each experiment. These variables include Number of Leaves (NoLVS) (both experiments), Rosette Height (RosHT) (first experiment) and Plant Height (HT) (second experiment), Number of Flowerheads and buds at 12 months ($NoF\&B_{12}$), Flowering stems ($FLOWERST_{12}$), Above Ground Biomass at 24 months (AGB_{24}) and Cumulative Fitness Index (CFI_{24}) (second experiment).

3.2.3.8 Linear Regression Analysis

The relationship between (i) Log Target Reproductive Population Size (LogTPS), (ii) Log Source Reproductive Population Size (LogSPS), (iii) Log Geographic Distance (LogGeogDist) and (iv) Environmental Distance (EnvDist) between populations and the difference in fitness between Target (local) and Source (foreign) plants for each individual fitness trait for both experiments was analysed using multiple (and single) linear regressions. A Stepwise ANOVA was used for model selection to identify the single variable, or combinations of variables that best explained the difference in fitness between local and foreign plants. Variables identified in the initial Stepwise ANOVA were subsequently analysed either as a simple linear regression (for a single variable) or multiple regression (for models with ≥2 explanatory variables). A general assumption of multiple linear regression analysis is that the number of data points (n) should be approximately 10 times the number of explanatory variables. Following this, a maximum of two explanatory variables were used in the regression models for analysis of the difference between the Target and Source populations (n =18).

3.3 Results

3.3.1 Local adaptation experiment 1

3.3.1.1 Germination (GERM)

Origin (Target (local) or Source (foreign)) had no significant effect on GERM including all population pairs ($P > 0.05$; Table 3.1) and for 14 of the 18 individual population pair comparisons (Figure 3.2 a; Appendix 3.1). Of the four significant population pairs, only one pair (LW-QB; 0.7 km) showed evidence of local adaptation, with a significant increase in GERM of 9.1 % in the local Target population (Figure 3.1 a). In contrast, a significant increase in GERM of between 10.5 – 16.6 % in the Source (foreign) population was observed for MA-BA (4.0 km), QB-RH (9.6 km) and CR-LW (15.2 km) (Figure 3.2 a), indicating greater GERM of foreign genotypes in these three population pairs.

3.3.1.2 Survivorship ($SURV_6$)

There were significant differences in $SURV_6$ between Target (local) and Source (foreign) populations for the overall analysis including all population pairs ($P < 0.001$; Table 3.1) and for 8 of the 18 individual population pair comparisons ($P < 0.001 - P = 0.025$; Figure 3.2 b; Appendix 3.1). Across all population pairs there was evidence of local adaptation, with a significant overall increase in $SURV_6$ of 7.2 (± 1.8) % in the local Target populations. Local adaptation was also observed in 6 individual population pairs spanning a range of geographic distances from 4.0 km (MA-BA) to 516.0 km (CF-SA) with increases in $SURV_6$ of between 15.0 – 49.2 % in the local Target population in these pairs (Figure 3.2 b). However, two population pairs (LW-QB and MJ-GB) showed evidence of foreign genotype advantage, with significantly greater $SURV_6$ (14.7 and 26.7 % respectively) in the foreign Source populations in these two pairs.

Table 3.1 Summary of the results of the overall GLM and REML analyses including all 18 population pairs examining differences between Target (local) and Source (foreign) plants for a range of traits across the life cycle in the first and second local adaptation experiments.

Analyses where Origin had a significant effect ($P < 0.05$) on plant performance are highlighted in bold. [1]Deviance ratio for GLM analysis (GERM, $SURV_3$, $SURV_6$, $FLOWERST_{12}$, CFI_{24})

	First local adaptation experiment			Second local adaptation experiment	
	Overall			Overall	
Fitness Trait	Wald/d.f.[1]	*P* value	**Fitness Trait**	Wald/d.f.[1]	*P* value
Seedling			**Seedling**		
Seed weight (SW) (mg)	**16.16**	**<0.001**	Seed weight (SW) (mg)	3.43	0.064
Germination (GERM)	0.01	0.932	Germination (GERM)	0.67	0.414
Seedling survivorhsip (3 months) ($SURV_3$)	**7.14**	**0.008**	Seedling survivorhsip (3 months) ($SURV_3$)	**10.53**	**0.001**
Seedling survivorhsip (6 months) ($SURV_6$)	**16.13**	**<0.001**	**Reproductive adult (12 months)**		
Number of leaves ($NoLVS_6$)	**14.92**	**<0.001**	Number of leaves ($NoLVS_{12}$)	1.43	0.231
Rosette height ($RosHT_6$) (mm)	1.36	0.244	Plant height (HT_{12}) (mm)	**6.46**	**0.011**
Leaf size (LFS_6) (mm^2)	0.02	0.882	Leaf size (LFS_{12}) (mm^2)	**4.59**	**0.032**
Index of plant size ($INDPS_6$)	2.93	0.087	Index of plant size ($INDPS_{12}$)	1.72	0.189
			Number of stems ($NoST_{12}$)	0.08	0.784
			Number of flowerheads and buds ($NoF\&B_{12}$)	0.04	0.839
			Flowering Stems ($FLOWERST_{12}$)	**5.38**	**0.021**
			Reproductive adult (24 months)		
			Above ground biomass (AGB_{24}) (mg)	**4.01**	**0.045**
			Cumultive fitness index (CFI_{24})	**3.91**	**0.048**

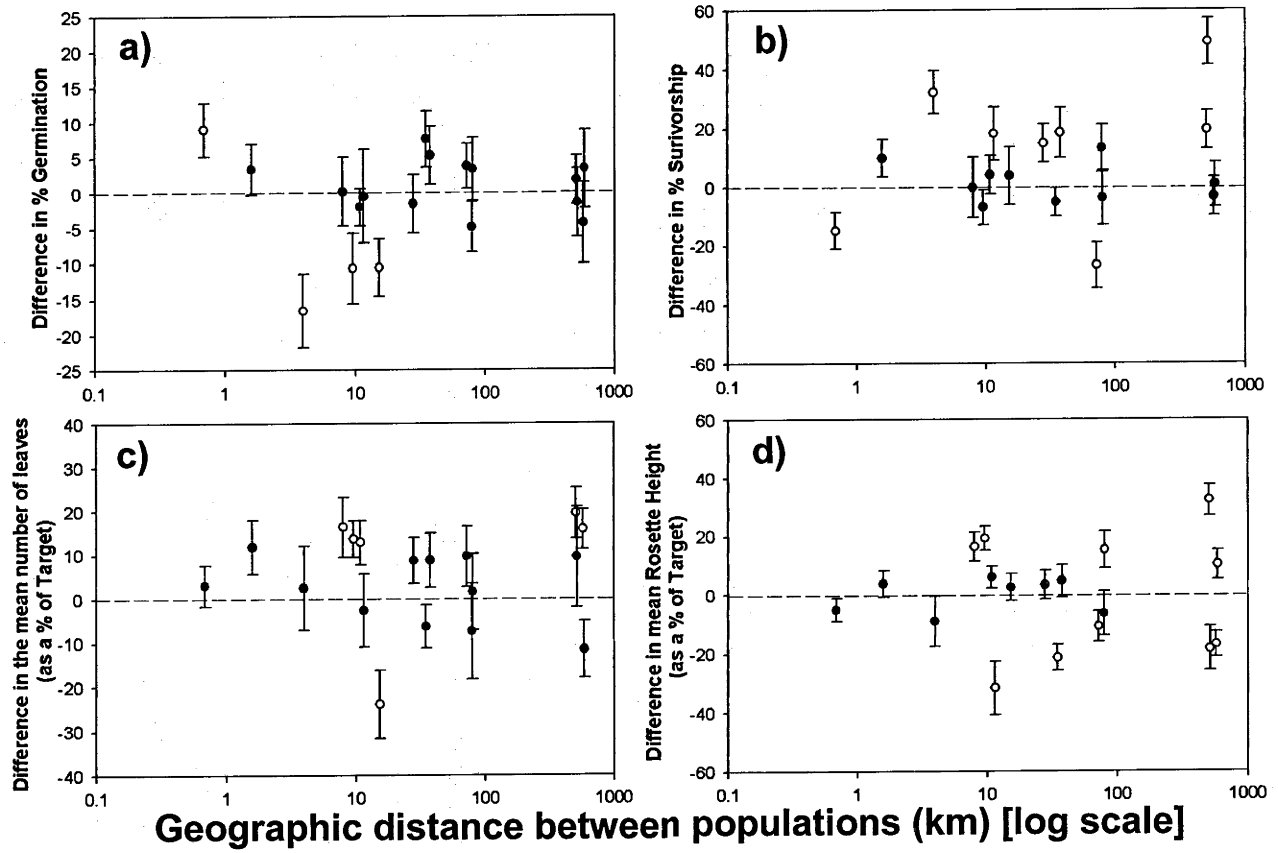

Figure 3.2 The percentage difference between the Target (local) and Source (foreign) populations (Target – Source) for a range of seedling fitness traits ((a) GERM, (b) $SURV_6$, (c) $NoLVS_6$ and (d) $RosHT_6$) in the first local adaptation experiment as a function of geographic distance.

For $NoLVS_6$ and $RosHT_6$ the difference between the Target and Source populations is expressed as a % of the Target population. Values above the dashed horizontal line (zero) represent local adaptation and those below represent foreign gene advantage. Population pairs where the difference between the Target and Source plants was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error. Note: axis scales vary for different fitness traits.

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3.3.1.3 Number of leaves ($NoLVS_6$)

The $NoLVS_6$ differed significantly between Target and Source populations including all population pairs ($P < 0.001$; Table 3.1) and for 6 of the 18 individual population pair comparisons representing a range of geographic distances from 8.0 – 575.1 km (Figure 3.2 c; Appendix 3.1). There was evidence of local adaptation in the overall analysis across all population pairs, with a significant overall increase of 5.9 (± 1.5) % in the $NoLVS_6$ in the Target (local) populations. Five individual population pairs (HH-MA (8.0 km), QB-RH (9.6 km), QB-SR (10.8 km), TR-SR (506.2 km) and GB-SA (575.1 km); Figure 3.2 c) showed significant local adaptation, with a 12.9 – 19.5 % increase in the $NoLVS_6$ in the local Target compared to the foreign Source population in these pairs. Only one population pair (CR-LW; 15.2 km) showed evidence of foreign genotype advantage with a 23.9 % increase in the $NoLVS_6$ in the Source (foreign) population (see Figure 3.2 c).

3.3.1.4 Rosette Height ($RosHT_6$)

Origin had no significant effect on $RosHT_6$ in the overall analysis including all population pairs ($P > 0.05$; Table 3.1) or for 8 of the 18 individual population pair comparisons (Figure 3.2 d; Appendix 3.1). However, for over half (10) of the individual population pairs, spanning a range of geographic distances, there were significant differences in $RosHT_6$ between Target and Source populations (Figure 3.2 d), with five population pairs showing evidence of local adaptation and five with evidence of foreign genotype advantage. Significant local adaptation, with an increase in $RosHT_6$ of between 10.1 – 32.2 %, was observed for HH-MA (8.0 km), QB-RH (9.6 km), GB-CC (79.9 km), TR-SR (506.2 km) and GB-TR (586.2 km) (see Figure 3.2 d). In comparison, the foreign genotype advantage observed in TR-SA (11.6 km), RH-CF (34.8 km), MJ-GB (71.9 km), CF-SA (516.0 km) and GB-SA (575.1 km) was similar in magnitude to that of local adaptation, with an increase in $RosHT_6$ of between 10.8 – 31.7 % in the Source (foreign) populations in these five pairs.

3.3.2 The interaction between local adaptation and environment

3.3.2.1 Germination (GERM)

For the overall analysis including all population pairs and for 11 of the 16 (69 %) individual population pair comparisons there was no significant interaction between the effect of Origin and Experiment ($P > 0.05$; see Figure 3.3 a; Appendix 3.3). For LW-QB (0.7 km), QB-RH (9.6 km), RH-CF (34.8 km), GB-CC (79.9 km) and CF-SA (575.1 km) (Figure 3.3 a), however, there was a significant interaction between Origin and Experiment, suggesting that, for these five population pairs, the effect of Origin on germination varied between the two experiments. Yet when the two experiments were analysed independently (see Appendix 3.1 local adaptation experiment 1 (LA EXP 1) and Appendix 3.2 local adaptation experiment 2 (LA EXP 2)) there were no population pairs with a significant interaction where the difference in germination between the Target and Source populations was significantly different from zero in both experiments (e.g. LW-QB: Difference in GERM significant in LA EXP 1, but not in LA EXP 2. GB-SA: Difference in GERM not significant in LA EXP 1, but significant in LA EXP 2). This suggests that the differences between experiments in these pairs could be due to random fluctuations around zero in one of the experiments, rather than consistent differences between LA EXP 1 and 2 in the effect of plant Origin on GERM.

3.3.2.2 Survivorship ($SURV_3$)

For the overall analysis including all population pairs and for 13 of the 16 individual population pair comparisons there was no significant interaction between Origin and Experiment ($P > 0.05$; see Figure 3.3 b; Appendix 3.3), indicating that, for the majority of population pairs (81 %) and in the overall analysis, Origin had a similar effect on $SURV_3$ in the first and second local adaptation experiments. LW-QB (0.7 km), HH-MA (8.0 km) and MJ-GB (72.0 km) were the only three population pairs with a significant interaction ($P < 0.05$; Figure 3.3 b) between Origin and Experiment. However, like for GERM, when each experiment was analysed separately (see Appendix 3.1 LA EXP 1 and Appendix 3.2 LA EXP 2) there were no population pairs with a significant interaction in which the difference in $SURV_3$ was significantly different from zero in

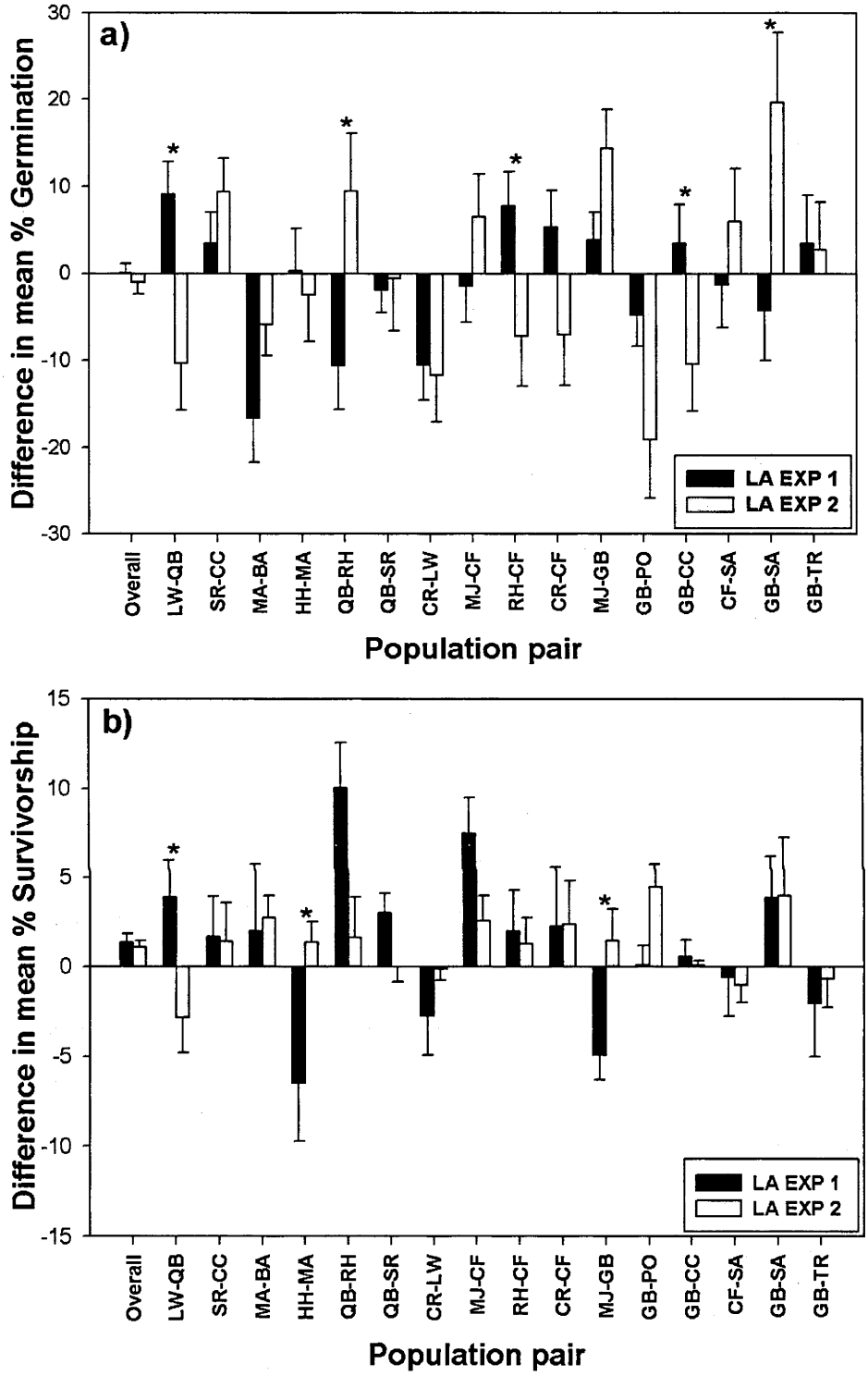


Figure 3.3 A comparison of the difference in germination (GERM) and seedling survivorship at 3 months ($SURV_3$) between the Target (local) and Source (foreign) populations for the first (LA EXP 1) and second (LA EXP 2) local adaptation experiments across all population pairs (Overall) and for the 16 individual population pair comparisons.

* Population pair with a significant interaction ($P < 0.05$) between Origin (local or foreign) and Experiment (Origin.Experiment). Population pairs are listed in order of increasing geographic distance between populations.

both experiments (e.g. HH-MA and MJ-GB: Difference in $SURV_3$ significant in LA EXP 1, but not in LA EXP 2). Furthermore, for LW-QB, the difference in $SURV_3$ between the Target (local) and Source (foreign) populations was not significantly different from zero in either LA EXP 1 or 2. As for GERM, this suggests that the observed variation between experiments in the difference in $SURV_3$ for LW-QB, HH-MA and MJ-GB could be the result of variability around zero, rather than a change in the effect of Origin between the two experiments.

3.3.3 Repeated Measures

Across all population pairs there was no significant interaction between Origin and Time (12 and 24 months) for the number of leaves (NoLVS), leaf size (LFS), plant height (HT), index of plant size (INDPS), number of stems (NoST) and number of flowerheads and buds (NoF&B) (Appendix 3.4) indicating that, including all population pairs, plant Origin had a similar effect on plant performance at 12 and 24 months. There was, however, variability between population pairs in the results of the repeated measures analysis (see Appendix 3.4), even though a significant interaction between Origin and Time was only observed in 2 – 4 of the 18 population pairs across a range of traits. For example, for NoLVS there was a significant interaction between Origin and Time in only three population pairs (HH-MA, GB-PO and GB-CC; see Appendix 3.4), while for the NoF&B, 4 of the 18 population pairs (SR-CC, RH-CF, GB-PO and SR-TR) showed a significant difference in the effect of plant Origin on reproductive output at 12 and 24 months. Therefore, considering the results of the overall analysis and that, across a range of traits, only a small percentage (11 – 22%) of the individual population pairs showed a significant interaction between Origin and Time, only results for the 12 month time period will be presented for NoLVS, LFS, HT, INDPS, NoST and NoF&B, since these results represent the effect of Origin on plant performance at 24 months.

3.3.4 Local adaptation experiment 2

3.3.4.1 Germination (GERM)

There was no significant difference between Target (local) and Source (foreign) populations in percent germination (GERM) across all population pairs ($P > 0.05$; Table 3.1) and for 13 of the 18 individual population pairs (Figure 3.4 a; Appendix 3.2). However, significant local adaptation was observed in three population pairs spanning a

range of geographic distances (SR-CC (1.5 km), MJ-GB (71.9 km) and GB-SA (575.1 km)) with a significant increase in GERM of 9.4 – 19.7 % in the local Target population in these three pairs (Figure 3.4 a). In contrast, a significant foreign genotype advantage was found in two population pairs (CR-LW (15.2 km) and GB-PO (78.9 km)) with a significant increase in GERM in the Source populations for CR-LW and GB-PO of 11.7 and 19.1 % respectively. These results suggest that, although Origin had a significant effect on GERM in approx. 25 % of population pairs, for the majority of population pairs and in the overall analysis GERM was not significantly different between the Target and Source populations.

3.3.4.2 Seedling survivorship ($SURV_3$)

Significant local adaptation was observed for seedling survivorship ($SURV_3$) in the overall analysis including all population pairs (Table 3.1) and for 2 of the 18 individual population pair comparisons (MA-BA (3.4 km) and GB-PO (78.9 km) (Figure 3.4 b; Appendix 3.2), even though for the majority of individual population pairs, Origin had no effect on $SURV_3$ (Figure 3.4 b). For the overall analysis, the local Target populations showed an overall increase in $SURV_3$ of 1.1 (± 0.3) % while for the two significant population pairs there was an increase in $SURV_3$ of 2.7 and 4.4 % in the local Target population for MA-BA and GB-PO respectively (see Figure 3.4 b).

3.3.4.3 Number of leaves ($NoLVS_{12}$)

Origin (Target (local) or Source (foreign)) had no significant effect on the number of leaves at 12 months ($NoLVS_{12}$) for the overall analysis ($P > 0.05$) (Table 3.1) and for 10 of the 18 individual population pair comparisons (Figure 3.4 c; Appendix 3.2). Of the 8 significant population pairs, half showed evidence of local adaptation (i.e. SR-CC (1.5 km), CR-LW (15.2 km), MJ-CF (27.8 km) and RH-CF (34.8 km); Figure 3.4 c) while the other four population pairs showed evidence of foreign genotype advantage (i.e. LW-QB (0.7 km), MA-BA (4.0 km), MJ-GB (71.9 km) and GB-PO (78.9 km); Figure 3.4 c). The magnitude of local adaptation and foreign genotype advantage was also similar for the significant population pairs, with a 10.5 – 31.7 % increase in the $NoLVS_{12}$ in the local Target population for pairs with significant local adaptation, while a 13.8 – 25.3 % increase in the $NoLVS_{12}$ in the foreign Source populations was observed for the four pairs with significant foreign genotype advantage.

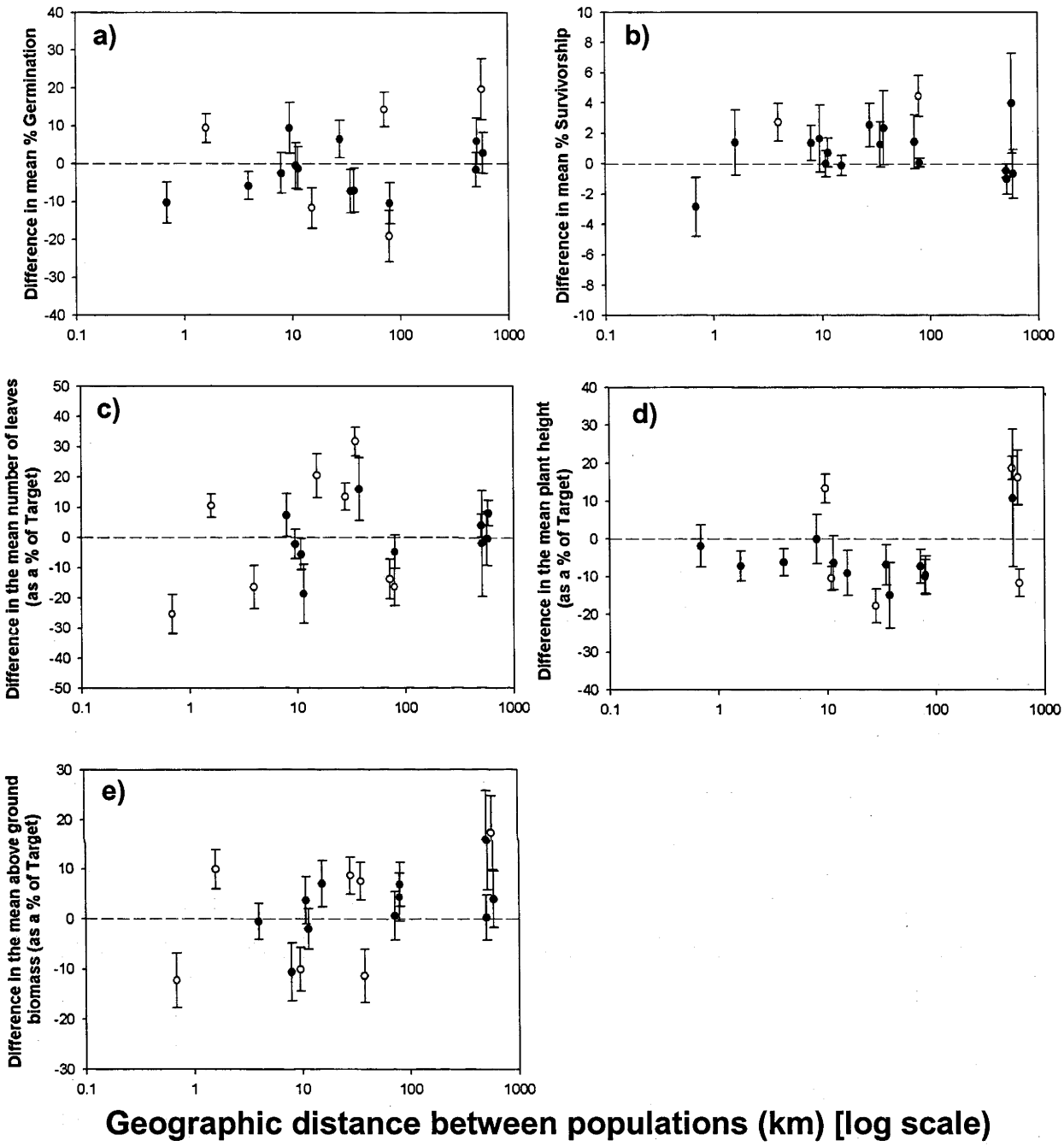


Figure 3.4 The percentage difference between the Target (local) and Source (foreign) populations (Target – Source) for (a) germination (GERM), (b) seedling survival ($SURV_6$), (c) number of leaves at 12 months ($NoLVS_{12}$), (d) mean plant height at 12 months (HT_{12}) and (e) above ground biomass at 24 months (AGB_{24}) in the second local adaptation experiment as a function of geographic distance.

Values above the dashed horizontal line (zero) represent local adaptation and those below represent foreign genotype advantage. Population pairs where the difference between the Target and Source plants was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error. Note: axis scales vary for different fitness traits.

3.3.4.4 Plant Height (HT_{12})

Origin had a significant effect on plant height at 12 months (HT_{12}) for the overall analysis including all population pairs (P = 0.011; Table 3.1) and for 7 of the 18 individual population pairs (Figure 3.4 d; Appendix 3.2). For the overall analysis, there was a significant foreign genotype advantage with an overall increase in HT_{12} of 3.3 (± 1.3) % in the foreign Source populations. However, for the individual population pair comparisons there was evidence of both local adaptation and foreign genotype advantage, with 3 population pairs showing local adaptation and 4 displaying foreign genotype advantage (Figure 3.4 d). For the 3 pairs with significant local adaptation (QB-RH (9.6 km), SR-TR (506.2 km) and GB-SA (575.1 km)) there was an increase in HT_{12} of between 13.3 – 16.2 % in the local Target population. In comparison, the foreign genotype advantage was similar in magnitude and ranged from a 10.0 % (GB-PO; 78.9 km) to 17.7 % (MJ-CF; 27.8 km) increase in HT_{12} in the foreign Source populations.

3.3.4.5 Above ground biomass (AGB_{24})

There was evidence of significant local adaptation for above ground biomass (AGB_{24}) in the overall analysis including all population pairs (P = 0.045; Table 3.1) and for 4 of the 18 individual population pairs, representing a range of spatial scales from 1.5 (SR-CC) to 575.1 km (GB-SA) (Figure 3.4 e; Appendix 3.2). For the overall analysis there was a significant increase in AGB_{24} of 2.5 (± 1.3) % in the local Target populations, while for the individual population pairs the percentage increase in AGB_{24} in the local Target population ranged from 7.5 (RH-CF) – 17.3 (GB-SA) %. Conversely, significant foreign genotype advantage was observed for AGB_{24} in three population pairs (LW-QB (0.7 km), QB-RH (9.6 km) and CR-CF (37.5 km)) with a significant increase in biomass of between 10.1 and 12.3 % in the foreign Source populations in these three population pairs (Figure 3.4 e).

3.3.4.6 Reproduction (Number of flowerheads and buds ($NoF\&B_{12}$) and Percent of Flowering stems ($FLOWERST_{12}$)

In 14 of the 18 population pairs (Figure 3.5 a; Appendix 3.2) and for the overall analysis (P > 0.05; Table 3.1) Origin (Target (local) or Source (foreign)) had no significant influence on the mean number of flowerheads and buds at 12 months ($NoF\&B_{12}$). Of

the four significant population pairs, two pairs showed evidence of local adaptation with an increase of 30.1 and 36.3 % in the $NoF\&B_{12}$ for GB-TR (586.2 km) and GB-SA (575.1 km) respectively (see Figure 3.4 a). Foreign genotype advantage was observed in the remaining two significant population pairs GB-PO (78.9 km) and SR-TR (506.2 km) with an increase of 42.3 % (GB-PO) and 69.2 % (SR-TR) in the $NoF\&B_{12}$ in the foreign Source populations in these two pairs (Figure 3.5 a).

There was a significant difference between Target (local) and Source (foreign) populations in the mean percentage of flowering stems ($FLOWERST_{12}$) for the overall analysis including all population pairs (P = 0.021; Table 3.1) and for 3 of the 18 individual population pairs (RH-CF (34.8 km), SR-TR (506.2 km) and GB-SA (575.1 km); Figure 3.5 b). An overall foreign genotype advantage was observed in the analysis including all population pairs, with an increase of 2.3 (± 1.0) % in mean $FLOWERST_{12}$ in the foreign Source populations. There was also evidence of foreign genotype advantage in all 3 significant population pairs, with an increase in mean $FLOWERST_{12}$ of between 8.4 and 14.6 % in the local Source populations in these three pairs (Figure 3.5 b).

3.3.4.7 Cumulative fitness (CFI_{24})

Considering plant fitness across the lifecycle from germination through to adult growth and reproduction (cumulative fitness), Origin (Target (local) or Source (foreign)) had a significant effect on cumulative fitness (CFI_{24}) including all population pairs (P = 0.046; Table 3.1) and for 4 of the 18 individual population pairs (P = 0.016 – P= 0.046; Figure 3.5 c; Appendix 3.2). For the analysis including all population pairs, an overall foreign genotype advantage was observed, with an increase in CFI_{24} of 10.1 (± 5.1) % in the foreign (Source) populations. Moreover, there was evidence of foreign genotype advantage in 3 of the 4 significant population pairs (i.e. RH-CF (34.8 km), CR-CF (37.5 km) and GB-PO (78.9 km)) with an increase in CFI_{24} of between 65.8 and 72.3 % in the Source populations in these pairs (Figure 3.5 c). Only one population pair, GB-TR (586.2 km), showed evidence of local adaptation, with a significant increase of 26.7 % in CFI_{24} in the local Target population for this population pair (Figure 3.5 c).

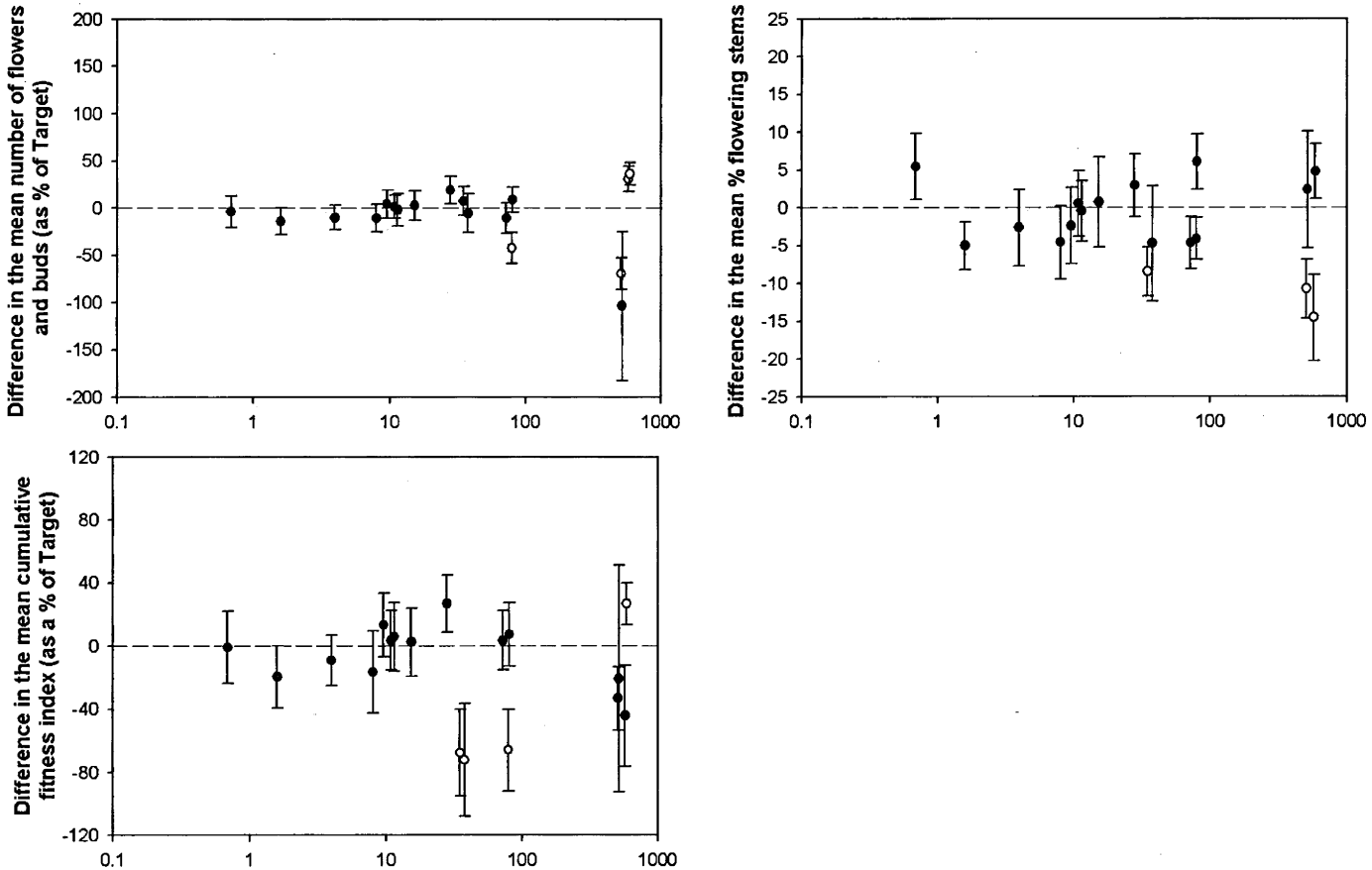


Figure 3.5 The percentage difference between the Target (local) and Source (foreign) populations (Target – Source) for reproduction ((a) NoF&B$_{12}$ and (b) FLOWERST$_{12}$) and cumulative fitness (CFI$_{24}$) at the adult reproductive life stage in the second local adaptation experiment as a function of geographic distance.

For NoF&B$_{12}$ and CFI$_{12}$ the difference between the Target and Source populations is expressed as a % of the Target population. Values above the dashed horizontal line (zero) represent local adaptation and those below represent foreign genotype advantage. Population pairs where the difference between the Target and Source plants was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error. Note: axis scales vary for different fitness traits.

3.3.5 Predicting local adaptation

For the majority of fitness traits in the first and second local adaptation experiments there was no relationship between the difference in fitness between the Target and Source populations (Target – Source) and geographic distance (GeogDist), environmental distance (EnvDist) and log Target (LogTPS) and log Source (LogSPS) population size. However, in the first local adaptation experiment, there was a negative relationship between the difference in survivorship ($SURV_6$) and LogSPS ($R^2 = 0.25$, $P = 0.02$; Table 3.2), with a significant decrease in local adaptation with increasing LogSPS. In the second local adaptation experiment, the difference in fitness between the Target and Source populations was significantly related to EnvDist and LogSPS for the $NoLVS_{12}$ ($R^2 = 0.42$, $P = 0.007$; Table 3.2) and to LogGeogDist for AGB_{24} ($R^2 = 0.21$, $P = 0.032$; Table 3.2). For the $NoLVS_{12}$, local adaptation was greatest in population pairs with small Source populations and large environmental distances, suggesting that both Source population size and environmental differentiation can influence the magnitude of local adaptation. For AGB_{24}, the difference in fitness between the Target and Source populations increased with geographic distance, indicating greater local adaptation at larger spatial scales.

3.4 Discussion

Local adaptation and patterns of adaptive differentiation are important considerations prior to the translocation of plants between populations. There was no difference in the performance of local and foreign genotypes for the majority of population pairs of *R. leptorrhynchoides*. There was, however, evidence of local adaptation and foreign genotype advantage in a number of population pairs for some seedling and adult growth and reproductive traits. The presence of both local adaptation and foreign genotype advantage was also reflected in the overall analysis including all population pairs, with local adaptation observed for seedling survivorship in both experiments and above ground biomass, while an overall foreign genotype advantage was found for plant height, flowering stems and cumulative fitness. Spatial scale, environmental distance and Target and Source population size had little power to predict patterns of local adaptation for most fitness traits, although for some traits ($SURV_6$, $NoLVS_{12}$ and

Table 3.2 Results of the single (and multiple) linear regression analysis examining the relationship between Log Source Reproductive Population Size (LogSPS), Environmental Distance (EnvDist) and Log Geographic Distance between populations (LogGeogDist) and local adaptation.

There was no significant relationship between any of the explanatory variables and the difference in fitness between Target (local) and Source (foreign) plants for fitness traits not included in the table. $P < 0.01$*** highly significant; $0.01 < P < 0.05$** significant; $0.05 < P < 0.1$* marginally significant

Fitness trait	R^2	P	Significant explanatory variable/s		
			Slope of relationship		
			LogSPS	GeogDist	EnvDist
First Local Adaptation Experiment					
Seedling (6 months)					
Survival ($SURV_6$)	0.25	0.02**	-		
Second Local Adaptation Experiment					
Reproductive Adult (12 months)					
Number of leaves ($NoLVS_{12}$)	0.42	0.007***	-		+
Reproductive Adult (24 months)					
Above ground biomass (AGB_{24})	0.21	0.032**		+	

AGB_{24}) some of the variability between population pairs was exaplained by Source population size, environmental distance and spatial scale.

3.4.1 Variability in local adaptation among population pairs

Variability between populations in the expression of local adaptation has important implications for understanding the process of adaptive evolution. The equivalent performance of local and foreign genotypes in the majority of population pairs, as well as evidence of both local adaptation and foreign genotype advantage suggests three potential adaptive states in *R. leptorrhynchoides*: (i) populations adapted to the local environment, (ii) populations with generalist genotypes that are potentially superior in a number of environments, and (iii) populations with high phenotypic plasticity that are able to adapt to a range of environments.

Superior performance of genotypes from a particular population or region is well recognised in forestry provenance trials (e.g. Pinyopusarerk et al. 1996; Sierra-Lucero et al. 2002; Turvey 1996) and has also been observed in a number of reciprocal transplant

experiments (e.g. Bischoff et al. 2006; Santamaria et al. 2003). For *R. leptorrhynchoides*, the superior performance of some Source populations in a number of environments (e.g. CF; RH-CF, CR-CF (CFI_{24})) may represent populations with generalist genotypes that are superior in a range of novel environments. Gene flow is an important evolutionary force that may counteract population divergence and constrain local adaptation through the transfer of genetic material between populations (Kawecki & Ebert 2004; Lenormand 2002; Slatkin 1987). The lack of local adaptation in populations of *R. leptorrhynchoides* may be due to high levels of gene flow among populations (see Young et al. 1999), since migration between populations can favour the development of plasticity over adaptive differentiation (Sultan & Spencer 2002). In addition, effective migration rates may be elevated in *R. leptorrhynchoides* due to its self-incompatibility system (see section 1.7.2). Selection should also favour generalist genotypes and plasticity when the temporal variability in selection pressures is greater than the spatial variability in environmental heterogeneity (Kawecki & Ebert 2004), such as in disturbance prone environments or in meta-populations with constant population turnover (e.g. *Chamaecrista fasciculata*, Galloway and Fenster (2000)). Populations of *R. leptorrhynchoides* may experience temporal variability in selection regimes, especially in relation to periodic disturbance by fire, which would lead to reduced local adaptation and selection for generalist genotypes. For this species, however, the major environmental component differentiating sites was edaphic composition, which is likely to be less temporally variable than climate. Yet high variability between different population pairs in the expression of local adaptation is not unexpected given that adaptive differentiation, drift and mutation are all factors that can act independently in different populations.

It is possible that maternal effects in response to environmental variability between populations may have contributed to differences in the performance of Target and Source populations. However, previous research on *R. leptorrhynchoides* found no significant fitness differences among plants from different populations grown in a common environment (A. Young, unpublished data), suggesting minimal environmentally induced maternal variation in this species. Maternal effects are also more likely to be important in early life-history characteristics (Mousseau & Fox 1997; Roach & Wulff 1987), yet in this study, the differences in performance of Target and

Source populations did not decrease across the life-cycle, suggesting that maternal effects are unlikely. Moreover, for the overall analysis and in most population pairs, seed weight had little influence on the majority of traits measured at both the seedling and adult reproductive life stage.

3.4.2 Variability between traits, elasticity values and cumulative fitness

There was variation in the expression of local adaptation and foreign genotype advantage between different fitness-related traits for a number of population pairs of *R. leptorrhynchoides* (see Figures 3.1 – 3.5). Yet for other population pairs (e.g. HH-MA, LW-SR, GB-CC and CF-SA) the absence of local adaptation was consistent across a range of traits. Variability between traits in the expression of local adaptation has been shown in a number of studies (e.g. Bischoff et al. 2006; Galloway & Fenster 2000; Joshi et al. 2001) and suggests potential variance in both gene expression and selection for local adaptation across the life cycle, and between different traits at a particular life stage. This variability also has important implications for predicting the long-term outcomes of translocation for population viability if there is differential expression of local adaptation in demographically important (i.e. high elasticity) traits.

Across all population pairs both local adaptation and foreign genotype advantage were observed in high elasticity traits (see Young et al. (2000b) for elasticity analysis), with local adaptation found for seedling survivorship ($SURV_{3,6}$) and above ground biomass (AGB_{24}) (as a surrogate for adult survivorship), while flowering stems ($FLOWERST_{12}$) showed an overall foreign genotype advantage. This suggests that translocation may have both positive and negative outcomes for mean population fitness, and that differences in performance between local and foreign genotypes may vary at different life stages. However, when fitness was assessed across the life cycle from germination through to reproduction (CFI_{24}), there was evidence of an overall foreign genotype advantage, indicating superior performance of the foreign Source populations. These results imply that even when translocating over large spatial scales (up to 600 km), foreign genotypes may have equivalent or greater performance than the local Target population.

3.4.3 Environmental variability in the expression of local adaptation

For the majority of population pairs, and in the overall analysis, local adaptation for germination (GERM) and seedling survivorship ($SURV_3$) was consistent between the two experiments (LA EXP 1 and LA EXP 2). For a number of population pairs, however, the effect of Origin varied between LA EXP 1 and 2, suggesting that environment may alter the expression of local adaptation. Temporal variability in the expression of adaptive differentiation had been documented in a number of studies (e.g. Galloway & Fenster 2000; Kindell et al. 1996; Macel et al. 2007; Rice & Mack 1991) and reflects the potential for genotype by environment interactions and temporal variation in selection. Yet as discussed in section 3.3.2, in no population pairs with a significant interaction was the difference in GERM and $SURV_3$ between the Target and Source populations significantly different from zero in both experiments. Therefore, the differences between experiments may be due to random variability around zero, rather than the differential expression of local adaptation in the two experiments. The primary difference in environment between the two experiments was water availability, since the same methodology was used to collect the field soil matrix in each population pair and there was little variability in climatic conditions between the two seasons (temperature and humidity recorded from data loggers, data not shown). Even though more stressful environments are often associated with a reduction in the expression of genetic effects (e.g. Bennington & McGraw 1995; Galloway & Fenster 2000; Kindell et al. 1996) the more stressful conditions of LA EXP 1 (reduced water availability for field transplants) may have increased the variability in seedling survivorship between populations in this experiment (Figure 3.2 b).

3.4.4 Predicting patterns of local adaptation

An association between adaptive population differentiation and spatial scale is driven by the expected increase in both genetic isolation and environmental heterogeneity with increasing geographic distance (Galloway & Fenster 2000; Montalvo & Ellstrand 2000). For *R. leptorrhynchoides*, local adaptation in above ground biomass (AGB_{24}) increased with geographic distance, suggesting greater adaptive differentiation at larger spatial scales. However, for a number of fitness traits (e.g. GERM, $NoLVS_{12}$ and AGB_{24}) significant local adaptation was observed at a range of spatial scales from 1.5 – 600 km. In previous studies of *C. fasciculata* adaptive differentiation was observed at both small

and large spatial scales, although local adaptation was more consistent over large geographic distances (Galloway & Fenster 2000). Greater local adaptation with increasing distance was also observed for *Carlina vulgaris* (Becker et al. 2006a), while for other species (e.g. *Lotus scoparius*, Montalvo & Ellstrand (2000) and *Aster amellus*, Raabová et al. (2007)) geographic distance had little power to predict patterns of local adaptation.

Enhanced performance of non-local genotypes at intermediate distances was observed in some population comparisons for *C. fasciculata*, suggesting that foreign genotypes may out-perform the local population (Galloway & Fenster 2000). This substantiates the results of foreign genotype advantage observed in this study, though, for *R. leptorrhynchoides*, superior fitness of foreign genotypes was observed over the full range of geographic distances (0.7 – 600 km). This spatial variability in patterns of local adaptation and foreign genotype advantage in *R. leptorrhynchoides* may result from the interplay of a number of evolutionary processes including genetic drift, gene flow and spatial and temporal heterogeneity in selection regimes, which are not necessarily associated with geographic distance between populations.

The predicted relationship between local adaptation and environmental distance is based on the role of environmental heterogeneity as the selective force in driving patterns of adaptive population differentiation, and has been demonstrated empirically for *Lotus scoparius* (Montalvo & Ellstrand 2000) and *Aster amellus* (Raabová et al. 2007). In the present study, local adaptation was only positively related to environmental distance for $NoLVS_{12}$. The absence of a more widespread association between environmental distance and local adaptation is surprising given the level of environmental differentiation between populations of *R. leptorrhynchoides*, particularly in soil characteristics (see Chapter 2). Local adaptation in response to specific soil conditions is well recognised (Ellis & Weis 2006; Sambatti & Rice 2006; Snaydon & Davies 1982; Wright 2007), although other components of the environment such as vegetation composition (e.g. Raabová et al. 2007), soil micro-organisms and/or biota (e.g. Lie et al. 1987; Macel et al. 2007) and local competitors (Bischoff et al. 2006; Kindell et al. 1996) may contribute to patterns of adaptive population differentiation. The absence of a more general relationship between environmental distance and local adaptation but may be

due to the contribution of other variables such as vegetation composition or competition to the expression of local adaptation, which were not included in either the growth experimental or environmental distance coefficient in this study. However, this study was designed to assess local adaptation specifically in relation to soil differences between populations, and climatic differences between the two zones (North and South; see chapter 2) and accordingly patterns of local adaptation are considered in the context of these two primary environmental components.

For seeding survivorship ($SURV_6$) and number of leaves at 12 months ($NoLVS_{12}$) the difference in fitness between Target and Source populations was negatively related to Source population size. Greater local adaptation in pairs with small Source populations may result from increased bi-parental inbreeding as population size declines. In this case, small Source populations, with greater levels of inbreeding, have reduced fitness compared to the local Target population, which is reflected as superior performance of local genotypes (local adaptation). This result suggests that population size is an important consideration when choosing potential source populations for translocation, and that small population processes, such as increased inbreeding (in this case bi-parental) and drift, may play an important role in determining overall fitness and adaptive potential in populations of *R. leptorrhynchoides*. An increase in the efficacy of selection in large populations is predicted to result in greater local adaptation in large compared to small populations (Linhart & Grant 1996). Furthermore, increased local adaptation in large populations was the key finding of a recent meta-analysis of local adaptation studies (Leimu & Fischer in review) and has been demonstrated for *Carlina vulgaris* (Jakobsson & Dinnetz 2005). For *R. leptorrhynchoides*, however, there was no relationship between Target population size and local adaptation indicating that, for this species, selection can produce adaptive differentiation in both large (e.g. SR-CC) and small (e.g. MA-BA) populations.

3.4.5 Conclusions

In summary, for the majority of population pairs in this study there was little evidence of adaptive population differentiation, although both local adaptation and foreign genotype advantage were observed in some pairs and in the overall analysis for a

number of fitness traits. Moreover, for those fitness traits that did show evidence of local adaptation or foreign genotype advantage, spatial scale, environmental distance and population size had limited power to explain variability between population pairs in the difference in fitness of Target and Source populations. Taken together, these results indicate that patterns of adaptive differentiation are determined by a complex range of processes that interact to produce a spectrum of fitness landscapes in populations of *R. leptorrhynchoides*, from adaptive differentiation to generalist genotypes superior in a range of novel environments. Furthermore, the absence of local adaptation in the majority of populations of *R. leptorrhynchoides* suggests that translocation of plants between populations would have no detrimental effects on population viability. This does, however, raise the question of the longer-term multi-generational implications of inter-population hyrbidisation in relation to outbreeding depression following the mating of local and foreign plants.

CHAPTER 4:
INTER-POPULATION HYBRIDISATION AND OUTBREEDING DEPRESSION IN FRAGMENTED POPULATIONS OF *Rutidosis leptorrhynchoides*

4.1 Introduction

Outbreeding depression is a critical issue in the restoration of threatened plant species where the mixing of genetic material between populations is considered as a potential management action to increase population numbers, augment genetic diversity and/or counteract inbreeding depression (Fenster & Galloway 2000a). It is also of central concern in delineating appropriate seed sourcing zones for revegetation (Hufford & Mazer 2003; Montalvo & Ellstrand 2001). Moreover, in an evolutionary context, understanding the fitness consequences of inter-population hybridisation can provide important insights into the evolutionary processes of adaptation, population divergence and speciation.

Currently, the majority of studies of outbreeding depression and the fitness consequences of inter-population hybridisation have only examined the fitness of hybrid progeny in the F_1 (see Table 1.1). Many of these studies found little or no evidence of outbreeding depression compared to the local parental population, suggesting that heterosis or hybrid vigour may dominate the fitness response of F_1 hybrid progeny. In comparison, the few studies that have examined outbreeding depression beyond the F_1 found either no detrimental fitness effects (Bailey & McCauley 2006; Erickson & Fenster 2006; Fenster & Galloway 2000b; Keller et al. 2000), or a decline in fitness in the F_2 (Fenster & Galloway 2000b; Keller et al. 2000), suggesting that in some cases heterosis may carry through to the F_2 and counteract any declines in fitness associated with the disruption of favourable epistasis. Interestingly, for the single study with F_3 progeny (Fenster & Galloway 2000b), the detrimental effects of outbreeding depression were largely delayed until the F_3, where hybrid breakdown was found to offset any remaining positive effects of heterosis. However, in this case, a subsequent study

(Erickson & Fenster 2006) found substantial recovery of fitness in the F_6, suggesting that the detrimental fitness effects of inter-population hybridisation may be temporary and that subsequent recombination can facilitate recovery from outbreeding depression. Therefore, comparing the fitness of hybrid progeny over a number of generations is essential to examining the relative importance of hybrid vigour vs. hybrid breakdown following inter-population hybridisation. The contribution of the different genetic mechanisms to the expression of outbreeding depression will be addressed explicitly in Chapter 5, while the current chapter will examine hybrid vigour and hybrid breakdown by assessing the fitness of F_1, F_2 and F_3 progeny compared to the local parental population.

As with inbreeding depression, the fitness consequences of inter-population hybridisation may vary across the life-cycle (Montalvo & Ellstrand 2001) and the expression of outbreeding may differ among fitness components (Edmands 2007). Many studies that have examined the fitness consequences of inter-population hybridisation have detected variation in the expression of outbreeding depression across different fitness traits (e.g. Bailey & McCauley 2006; Becker et al. 2006b; Fenster & Galloway 2000b; Fischer & Matthies 1997; Galloway & Etterson 2005; Heiser & Shaw 2006; Keller et al. 2000). Differences between traits across the lifecycle may be due to developmental (ontogenic) variation in the influence of the different underlying genetic mechanisms, and reflects the complexity of the expression of outbreeding depression (see also Chapter 5). However, it is largely unknown if fitness effects accumulate through the life-cycle resulting in the greatest expression of outbreeding depression in later life stages (Montalvo & Ellstrand 2001). It is, therefore, important to examine a range of fitness traits across the life-cycle to determine the relative importance of outbreeding depression following inter-population hybridisation.

Elasticity analysis can be used to identify fitness traits at different life stages which are most significant to demographic outcomes. In elasticity analysis, individual fitness traits are weighted by their demographic importance and contribution to population viability (Ouborg & Van Treuren 1997) and enable the interpretation of fitness components in a demographic context (Crone 2001). Considering the potential variation across different fitness traits, combining information on demography with the fitness response of

particular traits across the life-cycle over multiple generations will enable a more accurate assessment of the fitness outcomes of inter-population hybridisation and the potential effects for long-term population viability. As discussed in Chapter 3, previous demographic work on field populations of *R. leptorrhynchoides* (Young et al. 2000b) found that juvenile and adult survivorship, as well as adult reproductive characteristics had the highest elasticity values and therefore have a high contribution to population growth rate. Combining this information with the results of growth experiments examining the fitness of inter-population hybrids should provide important insights into the potential outcomes for mean population fitness and long-term viability following inter-population hybridisation in this species.

The level of genetic divergence between populations plays a central role in determining the expression of outbreeding depression (reviewed in Edmands 2002). A number of measures or surrogates of population divergence, including geographic and environmental distance between parental populations, can be used to predict outbreeding depression following inter-population hybridisation. Geographic distance can provide important insights into the spatial scale of adaptive evolution and has been used in a number of studies as a surrogate of evolutionary divergence (e.g. Edmands 1999; Fenster & Galloway 2000b). Due to the role of environmental heterogeneity as a selective force in driving adaptive population differentiation (Hedrick et al. 1976; Linhart & Grant 1996), an index of the environmental distance between parental populations also provides a relevant proxy of adaptive genetic distance (Montalvo & Ellstrand 2001) and the potential risk of outbreeding depression following inter-population hybridisation. In addition, population size may also explain patterns of hybrid fitness due to the increasing importance of drift and inbreeding in determining patterns of genetic variation and population differentiation as demographic population size declines and genetic effective population sizes are reduced (Barrett & Kohn 1991; Sherwin & Moritz 2000).

As discussed in Chapter 3, considering the potential consequences of mixing genetic material between populations first requires an assessment of patterns of local adaptation and the relative fitness of local and foreign plants. An evaluation of the risk of outbreeding depression, through an examination of the fitness outcomes for hybrid

progeny over a number of generations, is then required to enable an assessment of the consequences of inter-population hybridisation for long-term population viability. The aim of this chapter is to examine the fitness of F_1, F_2, F_3 and backcross progeny in 12 population pairs for fitness traits across the lifecycle, considering traits identified through elasticity analysis as having greatest demographic importance and in relation the geographical and environmental distance between populations. Specifically, I aim to address the following questions:

1. Are there differences in the fitness of F_1, F_2, F_3 and backcross progeny compared to the local parental population?
2. Is there variation in the fitness of F_1, F_2 and F_3 and backcross progeny across different fitness traits and does the fitness of hybrid progeny change throughout the lifecycle?
3. What are the fitness consequences for F_1, F_2 and F_3 and backcross progeny for traits of greatest demographic importance (i.e. high elasticity traits)?
4. Can population size and geographic and environmental distance between parental populations predict patterns of outbreeding depression across F_1, F_2 and F_3 backcross progeny?
5. Does the variability in progeny fitness in each cross type relate to Target population size?

4.2 Materials and methods

4.2.1 Seed collection

For all population pairs, seed from the Target and Source populations were collected from 1 – 3 open-pollinated inflorescences of 30 – 40 maternal plants in December 2001 – January 2002. Maternal plants were haphazardly chosen by walking through the population and collecting seed from plants distributed evenly throughout the population. For plants where the inflorescence had matured but the seed had not yet dehisced, tulle bags were placed over the inflorescence and seed collected after 2 – 3 weeks. This was done to ensure seed was sampled from throughout the population. From the potential pool of 30 – 40 maternal plants from each population, 12 – 18 maternal plants were chosen at random and removed for each Target and Source population in each

population pair. This ensured that different maternal families were used in each population pair that shared either a Target or Source population.

4.2.2 Crossing design and pollination experiment

4.2.2.1 Seed germination and glasshouse conditions

For each of the 12 population pairs, in August 2002, four seed from 12 – 18 maternal families were cold treated for 72 hours in a refrigerator set at approx. 5°C. Seeds were then germinated in 10 cm diameter petri dishes lined with filter paper in a growth cabinet maintained at 20° C with a 12 hour light/dark cycle. Germinated seedlings were subsequently transplanted into 10 cm diameter 0.5 L capacity pots containing a soil mix of 1/3 potting mix, 1/3 sand and 1/3 peat moss and grown in glasshouse conditions with temperatures maintained between 15 – 28°C. Crossing in this experiment was undertaken from November 2002 until January 2005. To ensure adequate flowering during the autumn and winter months over this time period, natural light was supplemented with artificial light to guarantee a 14-hour photoperiod. Once a plant was more than 4 months old it was transferred to a larger 20 cm diameter 1 L capacity pot. During the course of the crossing experiment, plants were re-potted every 8 – 12 months to encourage flowering and to avoid plants becoming root bound. In addition, a nutrient solution suitable for native plants (Hoagland (No. 2) solution; produced at the CSIRO Plant Industry Phytotron and adapted from Hewitt (1966)) was administered every 1 – 2 months.

4.2.2.2 Crossing design

The crossing design in each population pair is outlined in Table 4.1. All 12 population pairs involved control, F_1, F_2 and back-crosses to the Target and Source populations. In addition, for 5 population pairs, crosses were undertaken to the F_3 and included back-crosses to the Target and Source populations. Each population pair contained 12 – 15 maternal lines (with the exception of CF-SA where low numbers of SA plants meant that this population pair had only 10 maternal lines). Maternal lines were generated from from each plant from the Target population as outlined in Figure 4.1. A sub-sample of 6 maternal lines was used in the 5 population pairs that included F_3 progeny. For the 12 population pairs, the following pollination protocol was undertaken to produce the CONTROL and F_1 treatments. Each inflorescence was bagged on opening

Table 4.1 Crossing design for the 12 population pairs used in the outbreeding depression experiment

Population pair number	Population pair	CONTROL (TARGET x TARGET)	F_1 (TARGET x SOURCE)	F_2 (F_1 x F_1)	BC (F_1 x TARGET)	BC (F_1 x SOURCE)	F_3 (F_2 x F_2)	BC (F_2 x TARGET)	BC (F_2 x SOURCE)
1	LW-QB	LW x LW	LW x QB	F_1 (LW x QB) x F_1 (LW x QB)	F_1 (LW x QB) x LW	F_1 (LW x QB) x QB			
2	SR-CC	SR x SR	SR x CC	F_1 (SR x CC) x F_1 (SR x CC)	F_1 (SR x CC) x SR	F_1 (SR x CC) x CC	F_2 x F_2	F_2 x SR	F_2 x CC
3	MA-BA	MA x MA	MA x BA	F_1 (MA x BA) x F_1 (MA x BA)	F_1 (MA x BA) x MA	F_1 (MA x BA) x BA			
4	HH-MA	HH x HH	HH x MA	F_1 (HH x MA) x F_1 (HH x MA)	F_1 (HH x MA) x HH	F_1 (HH x MA) x MA	F_2 x F_2	F_2 x HH	F_2 x MA
5	CR-LW	CR x CR	CR x LW	F_1 (CR x LW) x F_1 (CR x LW)	F_1 (CR x LW) x CR	F_1 (CR x LW) x LW			
6	MJ-CF	MJ x MJ	MJ x CF	F_1 (MJ x CF) x F_1 (MJ x CF)	F_1 (MJ x CF) x MJ	F_1 (MJ x CF) x CF	F_2 x F_2	F_2 x MJ	F_2 x CF
7	RH-CF	RH x RH	RH x CF	F_1 (RH x CF) x F_1 (RH x CF)	F_1 (RH x CF) x RH	F_1 (RH x CF) x CF			
8	MJ-GB	MJ x MJ	MJ x GB	F_1 (MJ x GB) x F_1 (MJ x GB)	F_1 (MJ x GB) x MJ	F_1 (MJ x GB) x GB			
9	GB-PO	GB x GB	GB x PO	F_1 (GB x PO) x F_1 (GB x PO)	F_1 (GB x PO) x GB	F_1 (GB x PO) x PO	F_2 x F_2	F_2 x GB	F_2 x GB
10	SR-TR	SR x SR	SR x TR	F_1 (SR x TR) x F_1 (SR x TR)	F_1 (SR x TR) x SR	F_1 (SR x TR) x TR	F_2 x F_2	F_2 x SR	F_2 x TR
11	CF-SA	CF x CF	CF x SA	F_1 (CF x SA) x F_1 (CF x SA)	F_1 (CF x SA) x CF	F_1 (CF x SA) x SA			
12	GB-TR	GB x GB	GB x TR	F_1 (GB x TR) x F_1 (GB x TR)	F_1 (GB x TR) x GB	F_1 (GB x TR) x TR			

and remained bagged for the duration of the pollinations and then until the seed had matured and dehisced (4 – 5 weeks). (1) CONTROL treatment: One inflorescence from each of 2 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. (2) F_1 treatment: One inflorescence from each of 2 randomly chosen plants, one originating from the Target and one from the Source populations were gently brushed together to transfer pollen from the inner florets of one plant to the outer florets of the other. Crosses 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) so that although both reciprocal crosses contained the same proportion of nuclear genes, the seed from the maternal plant from the Target population contained the cytoplasmic background of the Target population. Conversely, for seed produced on the maternal plant from the Source population, the nuclear genes were expressed on the cytoplasmic background of the Source population. For each reciprocal cross-pollination, seed was collected from both plants and counted. If the number of seed on either side of the reciprocal cross was < 8 the cross was scored as unsuccessful and was repeated. This minimum threshold value was used to ensure adequate seed for the F_2 crossing treatment and for the final outbreeding depression growth experiment. *Rutidosis leptorrhynchoides* has a sporophytic self-incompatibility system (Young et al. 2000a) which meant that some cross-pollinations, particularly in small populations, did not produce viable seed presumably due to the sharing of *S*-alleles (see section 6.3). If < 8 seed were produced in the initial cross between the two randomly paired plants, the cross was first repeated to ensure that the low seed set was due to an incompatibility reaction, rather than environmental factors. However, incompatible crosses due to the matching of *S*-alleles usually produced 0 – 2 seed (see section 6.2). If the repeated cross

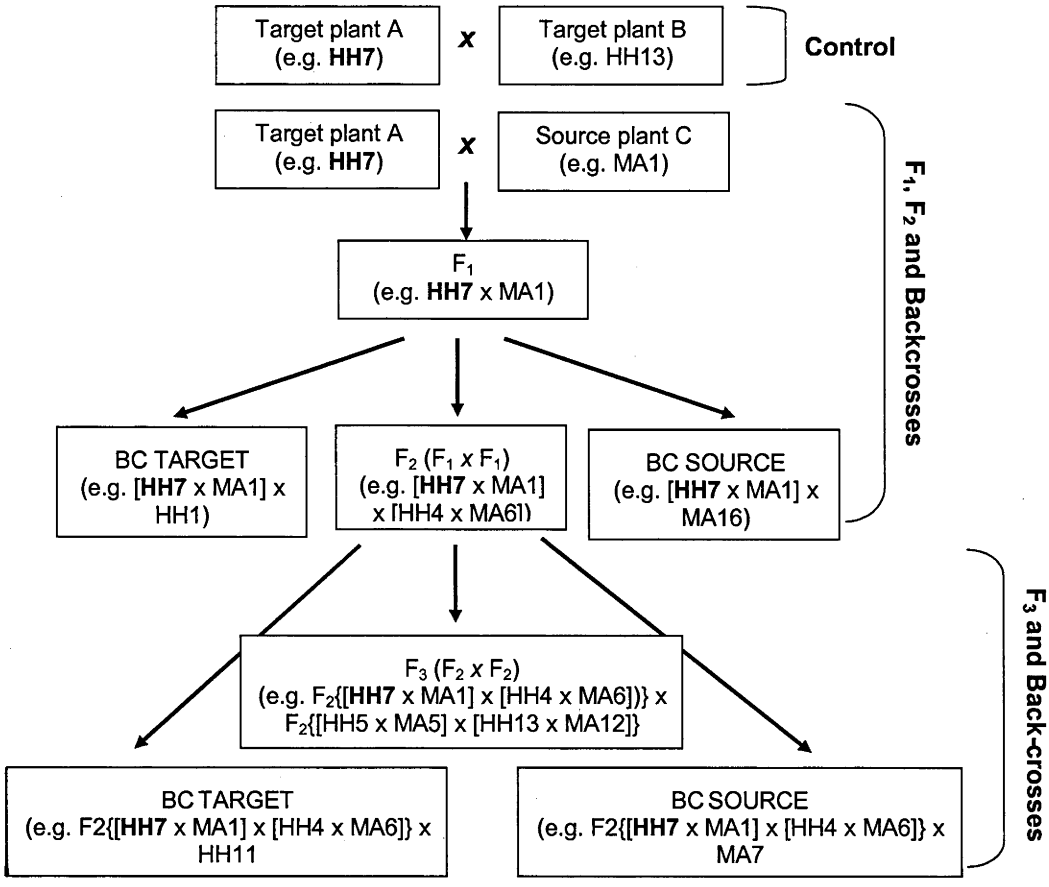


Figure 4.1 An example of the crossing structure for a single maternal line originating from the plant HH7 from the Target population in population pair HH-MA.

This maternal line had 8 cross-pollination treatments including CONTROL, F_1, F_2, $BC_{F1xTARGET}$, $BC_{F1xSOURCE}$, F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$.

produced less than 8 seed, the plant pair was re-randomised and the cross-pollination undertaken with a new pair of plants. This was done to ensure adequate sample size across the 12 maternal lines in each crossing treatment for the outbreeding depression growth experiment. For some random plant pairs, only one side of the reciprocal cross produced seed, which is possible due to dominance relationships between *S*-alleles. In this case, as long as at least 8 seed were produced, the cross was not repeated.

For all 12 population pairs, once the F_1 seed had been produced, 2 seed from both sides of each reciprocal cross (where available) from the 10 – 15 maternal lines were germinated in the glasshouse in separate 10 cm diameter 0.5 L capacity pots filled with the soil mix of 1/3 potting mix, 1/3 sand and 1/3 peat moss. Once seedlings had reached reproductive maturity (approximately 12 – 16 weeks in the glasshouse) a plant from each pair of reciprocal crosses was randomly chosen for use in the F_2 crossing treatment. This ensured that the F_2 crossing treatment contained a random sample of possible cytoplasmic backgrounds. Where only one side of the reciprocal cross produced adequate seed, the seedling from this maternal plant was automatically selected for the F_2 cross-pollination treatment.

For the 12 population pairs, the F_2 crossing treatment included three types of cross-pollinations: (1) F_2 (F_1 *x* F_1); F_1 plants in each maternal line were randomly paired with another F_1 plant to produce the F_2 progeny. In this crossing treatment, progeny contained the same average proportion of local and foreign genes (50:50) as the F_1, but had undergone recombination. (2) $BC_{F1xTARGET}$ (F_1 *x* TARGET); F_1 plants in each maternal line were randomly paired with plants from the Target population to produce the $BC_{F1xTARGET}$ progeny. In this treatment progeny contain a greater average proportion of local genes (75:25 local and foreign genes) and have undergone recombination. (3) $BC_{F1xSOURCE}$ (F_1 *x* SOURCE); F_1 plants were randomly paired with plants from the Source population to produce the $BC_{F1xSOURCE}$ progeny. In this treatment progeny have a reduced average proportion of local genes (25:75 local and foreign genes) and have undergone recombination. These three cross-pollination treatments were undertaken according to the crossing protocol outlined for the CONTROL and F_1 treatments.

For five population pairs (Table 4.1), sufficient F_2 seed had been produced by April 2004 to enable the generation of F_3 and F_3 backcross progeny. These population pairs were also chosen to ensure an even distribution of pairs with F_3 progeny across a range of spatial scales (see Table 2.1). The same protocol used to germinate and randomly choose plants for inclusion in the F_2 crossing treatments was utilised to generate plants for the F_3 cross pollination treatments. In these population pairs, 6 maternal lines were replicated across three crossing treatments: (1) F_3 (F_2 *x* F_2); F_2 plants in each maternal line were randomly paired with another F_2 plant to produce the F_3 progeny. In this treatment progeny contained the same average proportion of local and foreign genes (50:50) as the F_1 and F_2 treatments, but had undergone an additional round of recombination. (2) $BC_{F2xTARGET}$ (F_2 *x* TARGET); F_2 plants in each maternal line were randomly paired with plants from the Target population to produce the $BC_{F2xTARGET}$ progeny. In this treatment progeny contain a greater average proportion of local genes (75:25 local and foreign genes) and have undergone a subsequent round of recombination. (3) $BC_{F2xSOURCE}$ (F_2 *x* SOURCE); F_2 plants were randomly paired with plants from the Source population to produce the $BC_{F2xSOURCE}$ progeny. In this treatment progeny have a reduced average proportion of local genes (25:75 local and foreign genes) and have undergone an additional round of recombination. These three cross-pollination treatments were undertaken according to the crossing protocol outlined for the CONTROL, F_1 and F_2 cross-pollination treatments. The number of reciprocal crosses within each population pair ranged from 150 – 240, so that across all population pairs this experiment involved 2455 hand cross-pollinations.

4.2.3 Maternal lines and seed preparation

In all 12 population pairs, for a maternal line to be included in the outbreeding depression growth experiment the maternal line required progeny in each crossing treatment (CONTROL, F_1, F_2, $BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$). This criterion was extended to the F_3 for the 5 population pairs with F_3 cross-pollination treatments (F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$). In population pairs where > 12 maternal lines were available, 12 maternal lines were randomly chosen for inclusion in the outbreeding depression growth experiment. CF-SA was the only exception, with only 10 maternal lines available in this population pair and so all maternal lines were used.

For all cross-pollination treatments in each maternal line, one maternal plant was randomly chosen from the reciprocal pair for inclusion in the outbreeding depression growth experiment. This ensured that a random sample of possible cytoplasmic backgrounds was represented in each crossing treatment. Where only one side of the reciprocal cross was available, this maternal plant was automatically selected. From each randomly selected reciprocal cross, 12 seed (minimum of 4) were selected and weighed in bulk on a 4 dpl gram balance. This process was repeated for the 12 population pairs. Seeds were then cold treated in a refrigerator set at approx. 5°C for 72 hours.

4.2.4 Soil collection

For each population pair, soil was collected from the Target population. To minimise disturbance at each site and to ensure that soil used in this experiment represented an average soil profile for each site, soil was collected from the top 30 cm of the soil profile at 6 – 8 sub-sampling sites across the population. The amount of soil required from each Target population ranged from approximately 100 – 312 L. The soil from each site was then transported to CSIRO Plant Industry in Canberra and subsequently steam sterilised at 60 – 65°C for 45 minutes to reduced the viability of weed seeds within the soil-stored seed bank. To compensate for the loss of soil structure and permeability with disturbance, the sterilised soil was mixed in a ratio of 80:20 with river sand and then placed in 10 cm diameter 0.5 L capacity pots.

4.2.5 Experimental planting

The design of this growth experiment follows the protocol outlined for the second local adaptation experiment (see section 3.2.2.1). Progeny from each crossing treatment in each population pair were planted into soil from the Target population and grown in a common climate representative of the Target populations in the northern climate zone at CSIRO Plant Industry, Black Mountain laboratories in Canberra, ACT. As discussed in section 3.2.2.1, this enabled the local adaptation and outbreeding depression experiments to be undertaken in more controlled conditions while still providing an environment analogous to the Target population.

Planting was undertaken in autumn 2005 from the 28 April – 13 May. For each crossing treatment and maternal line in all population pairs, 1 – 3 seed were planted into each of four 10 cm diameter 0.5 L capacity pots containing soil from the Target population. This gave a total of 240 pots for the 6 population pairs (LW-QB, MA-BA, CR-LW, RH-CF, MJ-GB, GB-TR) with 12 maternal lines and CONTROL, F_1, F_2, $BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$ crossing treatments. CF-SA, which had 10 maternal lines, involved 200 pots. 312 pots were planted for the remaining 5 population pairs (SR-CC, HH-MA, MJ-CF, GB-PO, TR-SR) with 12 maternal lines for the crossing treatments CONTROL, F_1, F_2, $BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$ and 6 maternal lines for the crossing treatments F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$. Across all population pairs this gave a total of 3200 pots and 9397 seed.

For each maternal line, one pot containing seed from each crossing treatment was grouped together and the set of pots randomly allocated to one of 4 blocks (see Figure 4.2). The pots containing progeny from the different cross-pollination treatments for each maternal line were grouped together to ensure that all pots experienced the same micro-scale environmental conditions since each maternal line provided an independent assessment of the performance of the different crossing treatments compared to the control in each population pair. Within each maternal group the order of pots containing the different cross-pollination treatments was completely randomised. Each maternal group was allocated to a random row and column position within each block and each pot was identified only by a consecutive number corresponding to its random position. This was done so that subsequent monitoring could be undertaken without knowledge of plant identity. In parallel with the second local adaptation experiment (see section 3.2.2.4), pots were placed in the outdoor 'cold-frame' enclosure at CSIRO Plant Industry, Black Mountain Laboratories for the initial 6 months over autumn/winter from April – September 2005. After this time, plants were moved to benches in an outdoor enclosure (Figure 4.2). The block and row/column structure was maintained in both the 'cold-frame' and outdoor enclosures. In the 'cold-frame' enclosure natural precipitation was supplemented with hand-watering every 1 – 2 days as required, while in the outdoor enclosure an over-head watering system was used to supplement natural precipitation every 1 – 3 days (depending on the season) to ensure pots were not desiccated.

4.2.6 Monitoring and plant measurements

Germination and survivorship for the 3200 pots were scored weekly for the first 3 months and these data were used to assess maximum germination (GERM) and seedling survivorship ($SURV_3$). Seedling survivorship ($SURV_3$) was calculated on a family basis so that individuals were pooled across the four blocks and survivorship assessed for each maternal line. At 12 weeks, for pots where multiple seed had germinated, seedlings were culled to leave 1 seedling per pot. This plant was randomly chosen by selecting the seedling closest to the geometric centre of the pot. Where multiple seedlings had germinated in one block, but no seedlings from the corresponding crossing treatment and maternal line had germinated and/or survived to 3 months in another block, seedlings were transplanted between pots to maintain sample size and ensure maternal replication across the 4 blocks.

To assess the relative difference in the performance of the CONTROL and F_1, F_2, F_3 and BC_{TARGET} ($BC_{F1xTARGET}$ and $BC_{F2xTARGET}$) and BC_{SOURCE} ($BC_{F1xSOURCE}$ and $BC_{F2xSOURCE}$) treatments across the life-cycle, ten different variables including survivorship, growth and reproductive characteristics were measured at 4, 8 and 12 months. Fitness variables were measured at the three time periods as they correspond to three distinct life stages; 4 months represents the juvenile life stage, 8 months is the adult life stage and represents the period of peak vegetative growth, while the reproductive adult stage at 12 months represents the period of peak flowering and reproduction and enables an assessment of cumulative fitness across the life cycle from germination to reproduction. These fitness variables included;

1. Survivorship (SURV): For each Cross Type, individuals were pooled across the four blocks and survivorship assessed for each maternal line.
2. Plant height (HT): measured as the perpendicular height from the soil surface to the maximum point on the plant. At 4 months, plant height was measured as the maximum height of the basal rosette (RosHT).
3. Length of the longest stem (LLST): measured as the stem length from the rosette to the base of the inflorescence.

Figure 4.2 A photo of the outbreeding depression experiment in the outdoor enclosure at CSIRO Plant Industry.

The white box represents a set of eight plants from a single maternal line with Control, F_1, F_2, $BC_{F1xTARGET}$, $BC_{F1xSOURCE}$, F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$ progeny.

4. Number of leaves (NoLVS): This included counting all photosynthetically active leaves that were greater than 25 % expanded in the basal rosette and on the stems. Leaves were considered photosynthetically active if less than 50 % of the leaf had senesced.
5. Length of the longest leaf (LLLeaf).
6. Width of the longest leaf (WLLeaf).
7. Number of stems (NoST).
8. Number of flowering stems (FLOWERST).
9. Number of flowerheads (NoF).
10. Number of buds (NoBD).

At each life stage (juvenile (4 months), adult (8 months) and reproductive adult (12 months)) a number of additional fitness variables were generated using the above measurements including;

11. Leaf size (LFS): LLLeaf *x* WLLeaf (mm^2).
12. Index of Plant Size (INDPS): NoLVS *x* LFS.
13. Number of flowerheads and buds (NoF&B): NoINF + NoBD.

At 12 months, above and below ground biomass samples were obtained from all surviving plants. Below ground biomass was sampled by cutting the basal rosette at ground level and then washing the soil from the root mass. Above ground samples included all biomass above ground level including the basal rosette and stems. Both above (AGB) and below ground biomass (BGB) samples were oven-dried at 70°C for 3 days and then subsequently weighed individually on a 4 dpl gram balance. Total biomass for each plant (TB) was the addition of AGB and BGB.

A cumulative fitness index (CFI_{12}) was generated to quantify plant fitness across the lifecycle using Germination (GERM), the proportion of Flowering Stems at 12 months ($FLOWERST_{12}$) (as a proxy for reproductive output) and Total Biomass (TB_{12}). Total Biomass, as a measure of plant size, was used as a surrogate for reproductive adult survivorship because across all population pairs in this experiment the majority of plants that survived to 3 months ($SURV_3$) subsequently survived to reproduction ($SURV_{12}$) (minimum survival between Cross Types 87 – 98 % (LW-QB), maximum survival 100% in all Cross Types (GB-TR, HH-MA, MJ-GB, RH-CF, SR-CC and SR-TR)). For

each population pair, Total Biomass for each plant was converted to a proportion of the maximum biomass for that population pair. Therefore;

$CFI_{12} = (GERM \times FLOWERST \times TB_{PROPMAX})$, where

GERM = Proportion germinated,

FLOWERST = Proportion of flowering stems at 12 months, and

$TB_{PROPMAX}$ = Total biomass as a proportion of the maximum in each population pair

4.2.7 Statistical analysis

All analyses were undertaken using Genstat for Windows 9th Edition (VSN International, Oxford UK). For all variables at each life stage, exploratory data analysis was undertaken to assess the distribution of the data and examine the assumption of equal variance. At the juvenile life stage, two fitness traits $INDPS_4$ and LFS_4 were not normally distributed and were therefore log transformed prior to analysis. All other continuous variables were normally distributed and conformed to the assumption of equal variance. For all analyses outlined in the following section, residual plots were assessed to check the adequacy of the models and the assumptions of normality and homogeneity of the variance. Unless stated, all statistical tests were significance tested at $\alpha = 0.05$.

4.2.7.1 Preliminary analysis

A paired t-test was used to assess if transplantation at 12 weeks (see section 4.2.6) had a significant effect on subsequent plant growth across the life-cycle from 4 – 12 months. For this analysis, transplanted seedlings (n = 155) were paired with a non-transplanted seedling from the same maternal family and cross type and a one-sided paired t-test used to test the null hypothesis that the growth of non-transplanted seedlings was not greater than transplanted seedlings. Growth variables at 4, 8 and 12 months were assessed given that transplantation may have a greater impact on juvenile growth at 4 months and that this may decrease with age. For 12 of the 15 growth variables assessed, non-transplanted individuals had significantly higher growth compared to transplanted seedlings ($P < 0.001$). This was consistent across the life-cycle for all variables except index of adult size (INDPS) and number of leaves (NoLVS). For these two variables no significant difference ($P > 0.05$) in the growth of non-transplanted and transplanted seedlings was found at 8 and 12 months for INDPS ($INDPS_8$, $INDPS_{12}$) and at 12

months for NoLVS ($NoLVS_{12}$). Therefore, data for the 155 transplanted individuals was removed from the data set for all variables except $INDPS_8$, $INDPS_{12}$ and $NoLVS_{12}$ prior to analysis to avoid the introduction of variation into the data set.

4.2.7.2 Covariates

Due to the potential influence of seed size on fitness across the lifecycle (for full discussion see Chapter 3, section 3.2.3.2) seed weight was included as a covariate in both the GLM and REML models for germination and survivorship as well as in the growth analysis at 4, 8 and 12 months. However, for three population pairs (RH-CF, MJ-CF and GB-TR) there was a significant relationship between seed weight and cross type (see Table 4.3). An important assumption in Analysis of Covariance (ANCOVA) is independence between the factor and covariate. Therefore, due to collinearity between seed weight and cross type, seed weight was not fitted as a covariate for analysis in these three population pairs, but was included for the remaining 9 population pairs and for the overall analysis including all population pairs.

4.2.7.3 Repeated measures

A Split-Plot Repeated Measures ANOVA was used to examine if the effect of Cross Type on fitness traits varied across the life-cycle as measured at 4, 8 and 12 months (juvenile, adult and adult reproductive life stages). In this model seed weight was fitted as a covariate for the overall analysis across all population pairs and for the analysis for each population pair (except for population pairs RH-CF, MJ-CF and GB-TR; see section 4.2.7.2). Cross Type, Time and Cross Type *x* Time were fitted as main effects in the fixed model, while Maternal Line *x* Block, Plant Number and Date were fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model. Across the five variables included in this analysis (NoLVS, HT, LFS, INDPS and NoST) preliminary data analysis identified an unequal variance structure at 4 months compared to 8 and 12 months. Consequently, to maintain the assumption of equal variance, variables at the juvenile life stage (4 months) were excluded from the analysis. Therefore, the focus of the repeated measures analysis was to examine if the effect of Cross Type varied across the two adult life-stages (i.e. if there was a significant interaction between Cross Type and Time over these two time periods).

4.2.7.4 Generalised Linear Models - Logistic Regression

The effect of Cross Type on the binary response variables including Germination (GERM), Survivorship ($SURV_3$ and $SURV_{12}$) and the proportion of flowering stems ($FLOWERST_{12}$) was analysed using a Generalised Linear Model (GLM) (Logistic Regression) with a binomial distribution and logit link function. For these models Seed Weight (as a covariate; except for population pairs RH-CF, MJ-CF and GB-TR), Maternal Line *x* Block and Cross Type were fitted sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair (i.e. (Population pair/Maternal line) *x* Block). Accumulated Analysis of Deviance was used to assess the significance of the overall model and each term individually. Cross Type comparisons were assessed as significant if the difference in the predicted means was greater than the Least Significant Difference (LSD) (at $\alpha = 0.05$) and by comparing the *P* value for individual linear comparisons between the reference level (Control) and each Cross Type.

4.2.7.5 Generalised Linear Models (GLM) – General model

Differences between Cross Types in Cumulative Fitness Index (CFI_{12}) were assessed using a Generalised Linear Model (GLM) with a Poisson distribution and logarithm link function. Given that the CFI_{12} was not normally distributed, the relationship between the mean and variance (and m^2 and variance) was used to identify the appropriate distribution and choice of link function for this analysis. For this model Seed Weight (as a covariate; except for population pairs RH-CF, MJ-CF and GB-TR), Maternal Line *x* Block and Cross Type were fitted sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair (i.e. (Population pair/Maternal line) *x* Block). Accumulated Analysis of Deviance was used to assess the significance of the overall model and each term individually. Cross Type comparisons were assessed as significant if the difference in the predicted means was greater than the Least Significant Difference (LSD) (at $\alpha = 0.05$) and by comparing the *P* value for individual linear comparisons between the reference level (Control) and each Cross Type.

4.2.7.6 Restricted Maximum Likelihood (REML) Linear Mixed Models

For the normally distributed variables at the juvenile (4 months), adult (8 months) and reproductive adult (12 months) life stages (i.e. NoLVS, RosHT (4 months), HT (8 and 12 months), LFS, INDPS, NoST (8 and 12 months), NoF&B (12 months) and AGB, BGB and TB (12 months)) REML Linear Mixed Models were used to analyse the differences between Cross Types across all population pairs (Overall) and for each population pair independently. Although this fitness trial was designed as a balanced experiment, the loss of individuals during the course of the experiment due to plant mortality meant that the final data set for analysis was unbalanced. Consequently, a REML analysis of Linear Mixed Models was most appropriate to examine the effect of Cross Type on progeny fitness for a range of fitness traits across the lifecycle. The Control was chosen as the reference level for all analyses. For these models seed weight was fitted as a covariate for the overall analysis across all population pairs and for the analysis for each population pair (except for population pairs RH-CF, MJ-CF and GB-TR). Cross Type was fitted as the main effect in the fixed model, while Maternal Line *x* Block (which includes Maternal Line + Block + Maternal Line *x* Block) was fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model. Significant differences between Cross Types were assessed using the Least Significant Difference (LSD), which is defined (at $\alpha = 0.05$) as twice the standard error of the difference of the means for each comparison. Therefore, when the difference between the predicted means was greater than the LSD the comparison between Cross Types was considered significant. However, for all Cross Type comparisons, LSDs were only reported as significant if the *P* value for the global significance test was < 0.05.

A summary of the results of the REML analyses examining the effect of Cross Type on progeny fitness is presented for all fitness traits across the lifecycle in Appendix 4.1. However, due to significant correlations between most variables at each life stage ($r > 0.37$, $P < 0.05$; data not shown), more detailed results and analysis will only be presented for one representative variable at each life stage. These variables include Number of Leaves at 4 months ($NoLVS_4$), Plant Height at 8 months (HT_8), Total Biomass at 12 months (TB_{12}) and the Number of Flowerheads and buds at 12 months ($NoF\&B_{12}$). Further details on the difference between the Control (within Target

population cross) and each crossing treatment for a range of variables at each life stage can be found in Appendix 4.3 – 4.6.

4.2.7.7 Linear Regression Analysis

The relationship between (i) Log Target Reproductive Population Size (LogTPS), (ii) Log Source Reproductive Population Size (LogSPS), (iii) Log Geographic Distance (LogGeogDist) and (iv) Environmental Distance (EnvDist) between populations and the difference in fitness between the Control (within Target population cross) and hybrid progeny in each generation for each individual fitness trait across the lifecycle (seedling, juvenile, adult and reproductive adult life stage) was analysed using multiple (and single) linear regressions. A Stepwise ANOVA was used for model selection to identify the single variable, or combinations of variables, that best explained the difference in fitness between the Control and hybrid progeny in each generation. Variables identified in the initial Stepwise ANOVA were subsequently analysed either as a simple linear regression (for a single variable) or multiple regression (for models with ≥ 2 explanatory variables). A general assumption of multiple linear regression analysis is that the number of data points (n) should be approximately 10 times the number of explanatory variables. Following this, a maximum of two explanatory variables were used in the regression model for analysis of the difference between the Control and F_1, F_2 and BC ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) generations (n = 12), and a single variable only was used for analysis in the F_3 and BC generations ($BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$) (n = 5).

The relationship between the standard error of the means for each Cross Type (CONTROL, F_1, F_2, $BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) and Log Target Reproductive Population Size (LogTPS) for all fitness traits across the lifecycle was analysed using simple linear regression. This analysis was undertaken to examine if the variability in progeny fitness was related to the size of the Target population. Only CONTROL, F_1, F_2, BC generations ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) generations were included in this analysis due to the uneven distribution of LogTPS across population pairs with F_3 and BC ($BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$) progeny.

For all regression analyses, significance was tested at $\alpha = 0.05$, however, where $P < 0.01$ results were reported as highly significant and where $0.05 < P < 0.1$ results were reported as marginally significant.

4.3 Results

4.3.1 Repeated measures analysis

Across all population pairs there was a significant interaction between Cross Type and Time period for the number of leaves (NoLVS), leaf size (LFS), plant height (HT) and number of stems (NoST), but not for index of plant size (INDPS) ($P > 0.05$) (Appendix 4.2). This indicates that for these four fitness traits (NoLVS, LFS, HT and NoST), the effect of Cross Type on progeny fitness varied between these two life stages. There was, however, variability in the results of the repeated measures analysis between different population pairs (see Appendix 4.2). For example, for NoLVS, there was no significant interaction between Cross Type and Time for 10 of the 12 population pairs, with only HH-MA and GB-TR showing a significant difference in the effect of Cross Type on the NoLVS between 8 and 12 months. For plant height (HT) there was a significant interaction term in one of the twelve population pairs (MA-BA), while for NoST and LFS, one and four population pairs respectively showed a significant interaction between Cross Type and Time (Appendix 4.2). Considering the results of the overall analysis and the variability between population pairs in the interaction between Cross Type and Time, each fitness trait was analysed at each time period and the results of these analyses are presented separately for each life stage. However, due to the consistent effect of Cross Type on INDPS at 8 and 12 months results of the analysis for this variable are considered at the adult (8 month) life stage only.

4.3.2 Seedling

4.3.2.1 Germination (GERM)

Cross Type had a significant effect on GERM in the overall analysis including all population pairs ($P < 0.001$; Table 4.2)) and for 7 of the 12 individual population pair comparisons ($P < 0.001 - 0.029$; see Appendix 4.3). For the overall analysis, all generations except the F_1 showed significant heterosis (see Table 4.2). Heterosis was also observed in a number of individual population pairs, with RH-CF (34.8 km), MJ-

GB (71.9 km) and CF-SA (575.1 km) all showing a consistent increase in GERM of between 17 – 30 % in the F_2 and BC generations ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$), while F_2 (SR-TR; 506.2 km) and $BC_{F1xTARGET}$ (GB-PO; 78.9 km) progeny also showed significant heterosis (Figure 4.3 b, c and d). In the F_3 and BC generations heterosis was observed in 4 out of 5 population pairs for the $BC_{F2xTARGET}$ (Figure 4.3 f) and in a single population pair for both the F_3 and $BC_{F1xSOURCE}$ generations (Figure 4.3 e and g). In contrast, significant outbreeding depression was only observed in F_1 progeny in SR-TR (Figure 4.3 a) and F_2 progeny for GB-PO (Figure 4.3 b), suggesting that for the majority of population pairs, and for the overall analysis, hybrid progeny was either not significantly different or had greater GERM than progeny from the local parental population.

4.3.2.2 Survivorship ($SURV_3$)

There were significant differences between Cross Types for seedling survival at 3 months ($SURV_3$) across all population pairs ($P = 0.027$; Table 4.2) and for 2 of the 12 individual population pairs (LW-QB, $P = 0.012$; MJ-CF, $P = 0.008$; Appendix 4.3). Across all population pairs, significant heterosis was only observed in the $BC_{F2xTARGET}$ generation with the remaining Cross Types showing no difference in $SURV_3$ compared to the Control (see Table 4.2). For individual population pairs, significant heterosis was observed in F_2 and $BC_{F1xTARGET}$ progeny in LW-QB (6 and 8 % respectively) and within the BC generations ($BC_{F2xTARGET}$ (6 %) and $BC_{F2xSOURCE}$ (6%)) for MJ-CF. However, for the majority of individual population pairs (10/12) there were no significant differences between Cross Types in $SURV_3$ ($P > 0.05$; Appendix 4.3). The prevalence of no significant difference in $SURV_3$ between Cross Types and evidence of some heterosis in later generations indicates that for this demographically important trait, inter-population hybridisation may have either no effect or potentially increase seedling survivorship in some hybrid progeny compared to the local parental population.

4.3.3 Juvenile

4.3.3.1 Number of leaves ($NoLVS_4$)

The $NoLVS_4$ differed significantly between Cross Types including all population pairs ($P < 0.001$, Table 4.2) and for 3 of the 12 individual population pair comparisons representing a range of geographic distances (SR-CC (1.5 km) $P = 0.008$, RH-CF (34.8

km) P = 0.004 and GB-PO (78.9 km) P = 0.027; Appendix 4.4), with significant heterosis observed in some population pairs for all generations expect the F_1 and $BC_{F2xSOURCE}$ (Figure 4.4). For the overall analysis across all population pairs there was significant heterosis in the F_2, $BC_{F1xSOURCE}$, and $BC_{F1xTARGET}$, and within the F_3 with an increase in the $NoLVS_4$ of between 11 – 18 % in these generations (Table 4.2). For individual population pairs, F_2 and BC progeny within RH-CF showed consistent heterosis of up to 43 %, while for GB-PO significant heterosis of between 25 – 38 % was observed in the F_2 and F_3 as well as for $BC_{F1xTARGET}$ and $BC_{F2xTARGET}$ progeny (Figure 4.4 b – f; Appendix 4.4). Outbreeding depression was only observed in the F_1 for a single population pair (SR-CC) (Figure 4.4 a) and did not carry through to subsequent generations (Figure 4.4 b – g). These results suggest that, for the majority of population pairs, inter-population hybridisation had either no effect or increased the number of leaves at the juvenile life stage compared to the local parental population.

4.3.4 Adult (8 months)

4.3.4.1 Plant height (HT_8)

For 6 of the 12 population pairs (Appendix 4.5) and in the overall analysis (Table 4.2) Cross Type significantly influenced plant height at 8 months (HT_8). For the overall analysis, including all population pairs, there was significant heterosis for HT_8 in the F_1, F_2 and BC ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) generations, but no difference in HT_8 between the control and F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$ hybrid progeny (Table 4.2). For individual population pairs an increase in HT_8 of 22 – 28 % was observed in F_1 progeny for MJ-CF, RH-CF and MJ-GB (Figure 4.5 a). This heterosis response was also observed in the F_2 and $BC_{F1xSOURCE}$ progeny for both MJ-CF and RH-CF and in the $BC_{F1xTARGET}$ for RH-CF (Figure 4.5 b – d). For the five population pairs with F_3 progeny, only $BC_{F2xSOURCE}$ progeny within MJ-CF showed significant heterosis for HT_8 (Figure 4.5 g). HH-MA was the only population pair with consistent outbreeding depression in all hybrid progeny from the F_1 – F_3 (Figure 4.5 a – g; Appendix 4.5). Significant outbreeding depression for HT_8 was also observed in SR-TR within the $BC_{F1xSOURCE}$ (Figure 4.5 d) and $BC_{F2xTARGET}$ (Figure 4.5 f) generations. These results indicate that while there was variability in hybrid progeny fitness between population pairs, with some exhibiting heterosis and others outbreeding depression, for the majority

Table 4.2 Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control, as % of Control) for the overall analysis including all population pairs for a sub-set of fitness traits across the life-cycle.

Positive values are where the mean of each fitness trait in the crossing treatment (F_1, F_2, F_3 and BC's) was greater than the Control and represents the % increase in germination, plant growth, reproduction or cumulative fitness across all population pairs. Negative values are where the mean of each fitness trait in the crossing treatment was less than the Control and represents the % decrease in germination, plant growth, reproduction or cumulative fitness across all population pairs. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$).

	Fitness trait and life stage						
	Seedling		Juvenile (4 months)	Adult (8 months)	Reproductive Adult (12 months)		
Generation	Germination (GERM)	Survivorhsip ($SURV_3$)	Number leaves ($NoLVS_4$)	Plant height (HT_8)	Total biomass (TB_{12})	Number of flowerheads and buds ($NoF\&B_{12}$)	Cumulative fitness index (CFI_{12})
P value	**<0.001**	**0.027**	**<0.001**	**<0.001**	0.065	0.155	**<0.001**
F_1	1.7	1.0	-0.8	**7.7**	2.1	8.4	11.0
BC (F_1 x Source)	**6.0**	0.3	**11.7**	**5.8**	-0.7	11.9	**20.8**
F_2 (F_1 x F_1)	**6.8**	0.5	**15.0**	**6.9**	4.4	11.7	**23.1**
BC (F_1 x Target)	**9.1**	-0.2	**11.0**	**6.5**	2.1	6.1	**24.0**
BC (F_2 x Source)	**11.1**	1.5	5.3	1.6	-1.0	-5.0	8.0
F_3 (F_2 x F_2)	**16.4**	2.7	**18.0**	-1.9	0.3	3.0	**21.5**
BC (F_2 x Target)	**18.5**	**3.9**	0.6	1.1	1.0	-0.2	**32.4**

Figure 4.3 (on following page) The difference in mean percent germination between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.

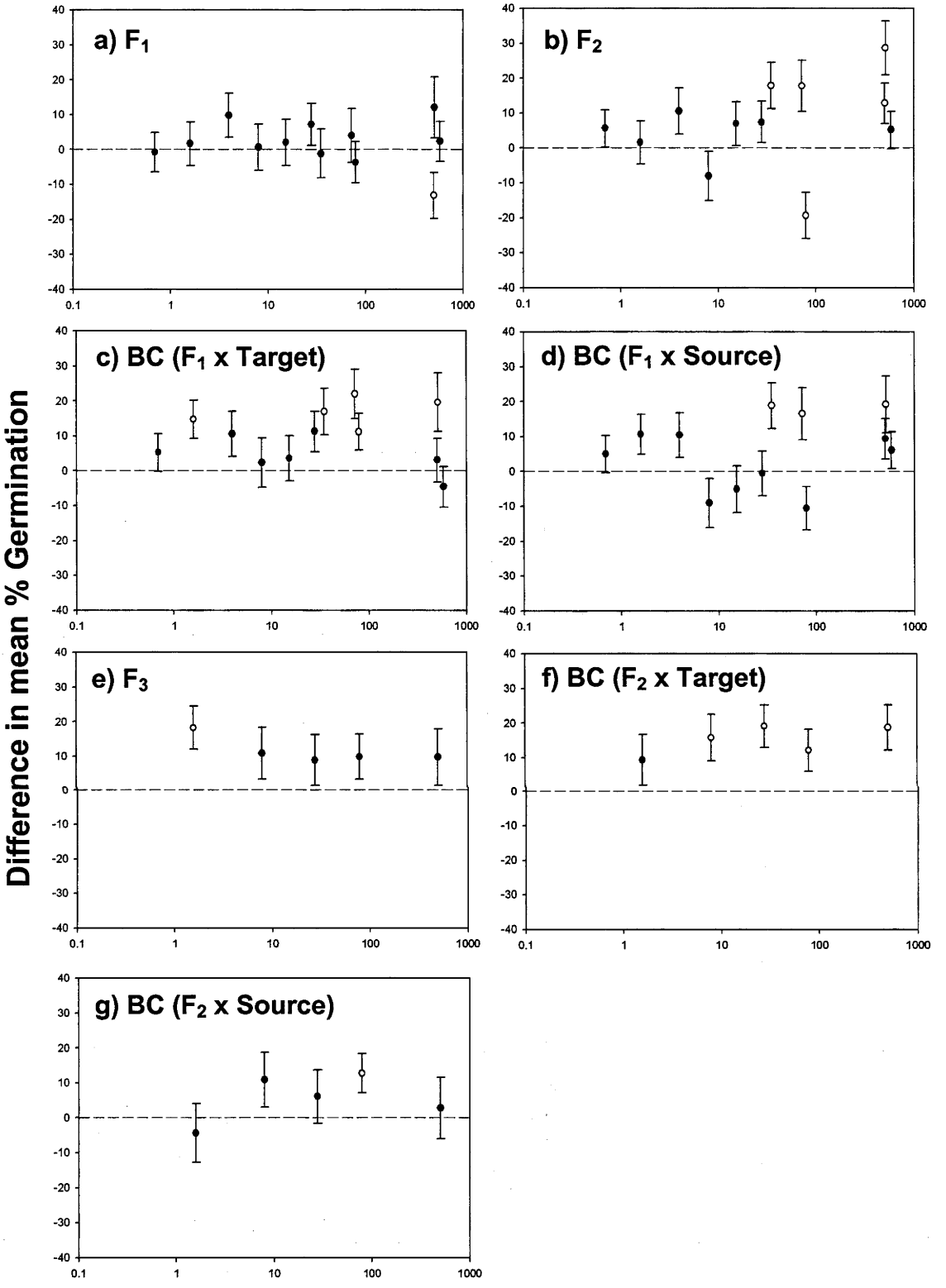
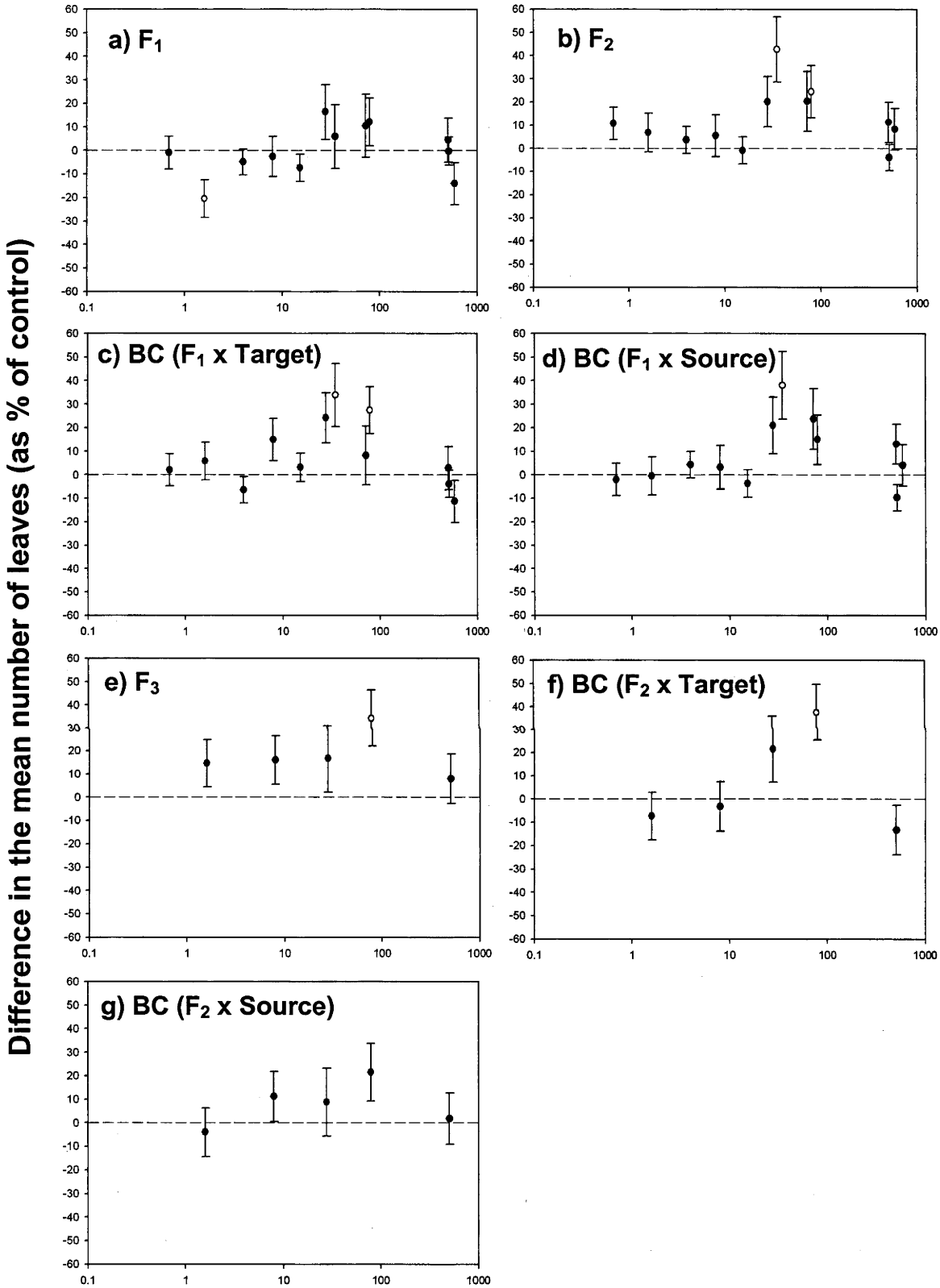

a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in mean % Germination
Geographic distance between populations (km) [log scale]

Figure 4.4 (on following page) The difference in the mean number of leaves at 4 months between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.


a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in the mean number of leaves (as % of control)
Geographic distance between populations (km) [log scale]

of population pairs there was no significant difference in HT_8 between hybrid progeny and the local parental population (Figure 4.5 a – g). In addition, for the overall analysis considering all population pairs, significant heterosis was observed for HT_8 in the F_1, F_2 and BC generations (Table 4.2).

4.3.5 Reproductive adult (12 months)

4.3.5.1 Total biomass (TB_{12})

For the overall analysis ($P > 0.05$; Table 4.2) and in the majority of population pairs (8/12) there was no significant difference in mean TB_{12} between the local parental population and hybrid progeny in the F_1, F_2, F_3 and BC generations (Figure 4.6 a – g). However, there were significant differences between Cross Types in mean TB_{12} for four population pairs spanning a range of geographic distances; SR-CC (1.5 km, $P = 0.031$), MJ-CF (27.8 km, $P = 0.039$), GB-PO (78.9 km, $P = 0.018$) and GB-TR (586.2 km, $P = 0.037$) (Appendix 4.6). For MJ-CF F_1, F_2 and $BC_{F1xTARGET}$ progeny showed a significant increase in mean TB_{12} of up to 16 % compared to the control (Figure 4.6 a, b and c). Significant heterosis was also observed for F_2 progeny in GB-PO (Figure 4.6 b), with an increase in mean TB_{12} of 11 % in F_2 hybrids. In contrast, for one population pair (SR-CC) there was consistent outbreeding depression in the F_1, F_2 and BC ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) generations (Figure 4.6 a – d) and also in the $BC_{F2xTARGET}$ generation (Figure 4.6 f). For this population pair, hybrid progeny showed a decrease in TB_{12} of up to 10 % compared to the local parental population.

4.3.5.2 Reproduction (Number of flowerheads and buds ($NoF\&B_{12}$))

For 8 of the 12 population pairs and for the overall analysis, inter-population hybridisation had no influence on the mean $NoF\&B_{12}$ compared to the local parental population (Table 4.2; Figure 4.7 a – g). However, there was significant heterosis in $NoF\&B_{12}$ for 3 of the 12 population pairs (CR-LW, GB-PO and GB-TR). For CR-LW and GB-PO there was variability between hybrid progeny, with the majority of crossing treatments showing no difference in the mean $NoF\&B_{12}$ compared to the local parental population, while significant heterosis of 34 % and 60 % was observed for $BC_{F1xTARGET}$ progeny within CR-LW (15.2 km; Figure 4.7 c) and $BC_{F1xSOURCE}$ progeny for GB-PO (78.9 km; Figure 4.7 d) respectively. In contrast, consistent heterosis in $NoF\&B_{12}$ of

Figure 4.5 (on following page) The difference in mean plant height at 8 months between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.

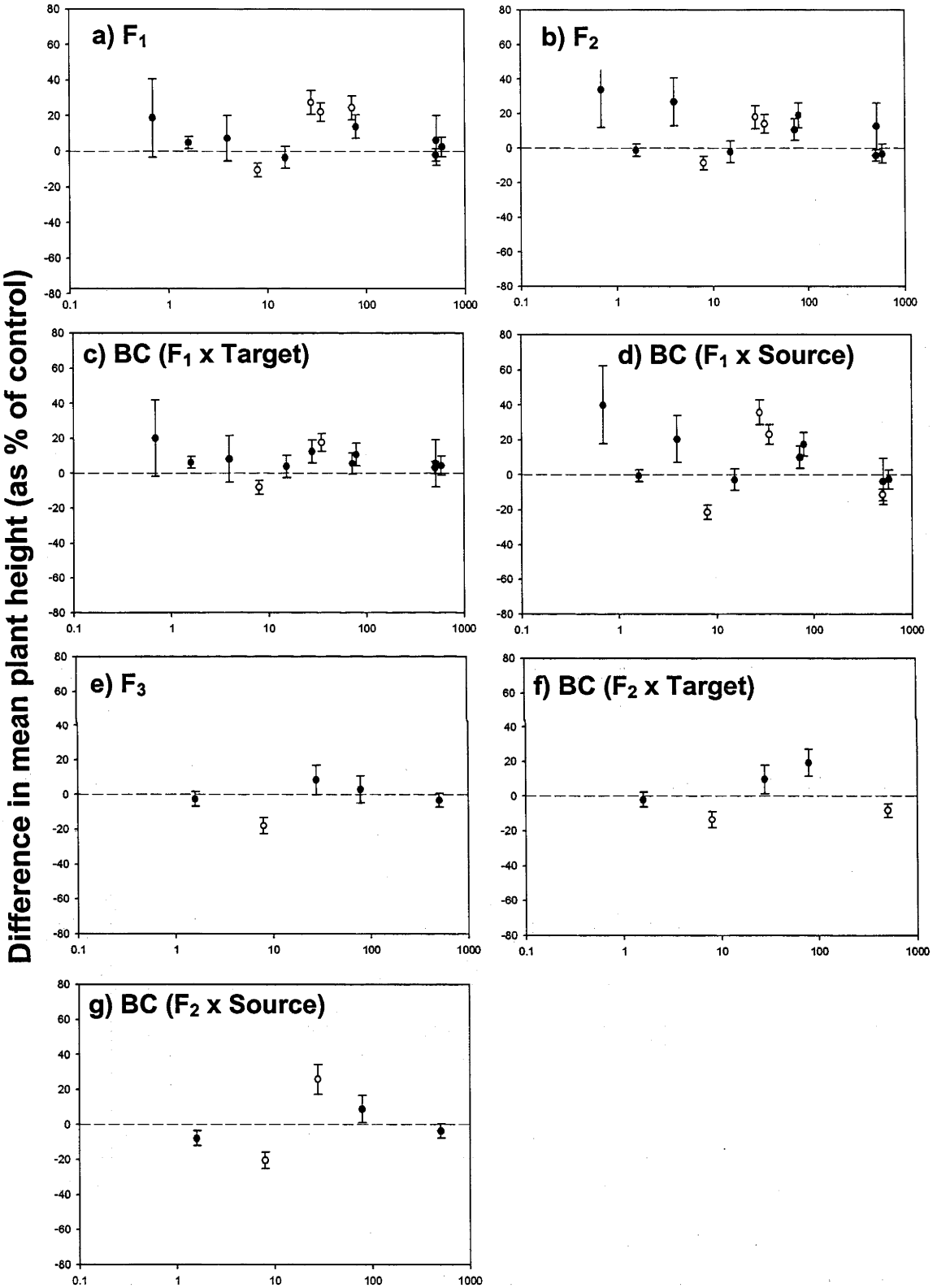

a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in mean plant height (as % of control)
Geographic distance between populations (km) [log scale]

Figure 4.6 (on following page) The difference in mean total biomass at 12 months between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.

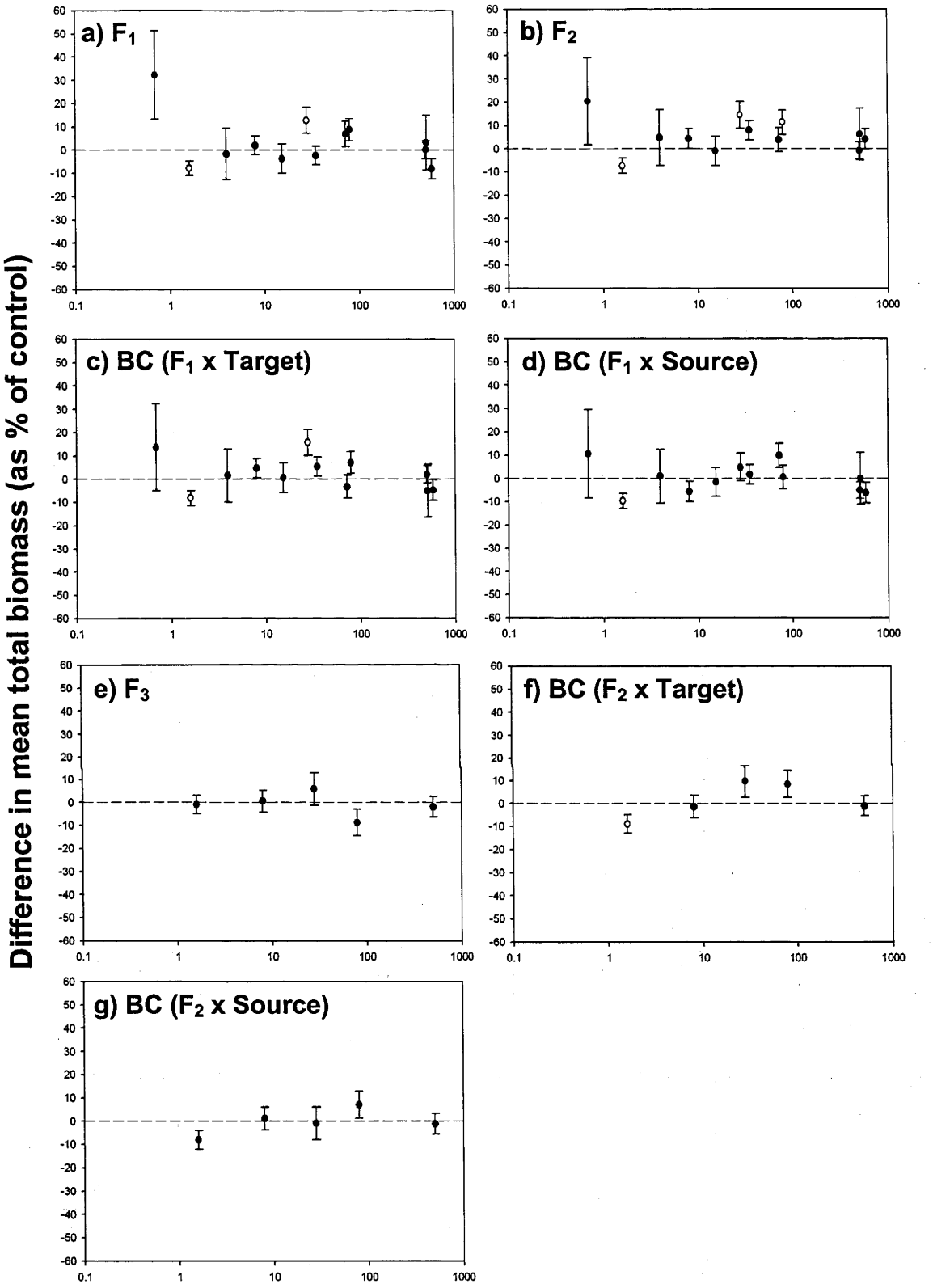
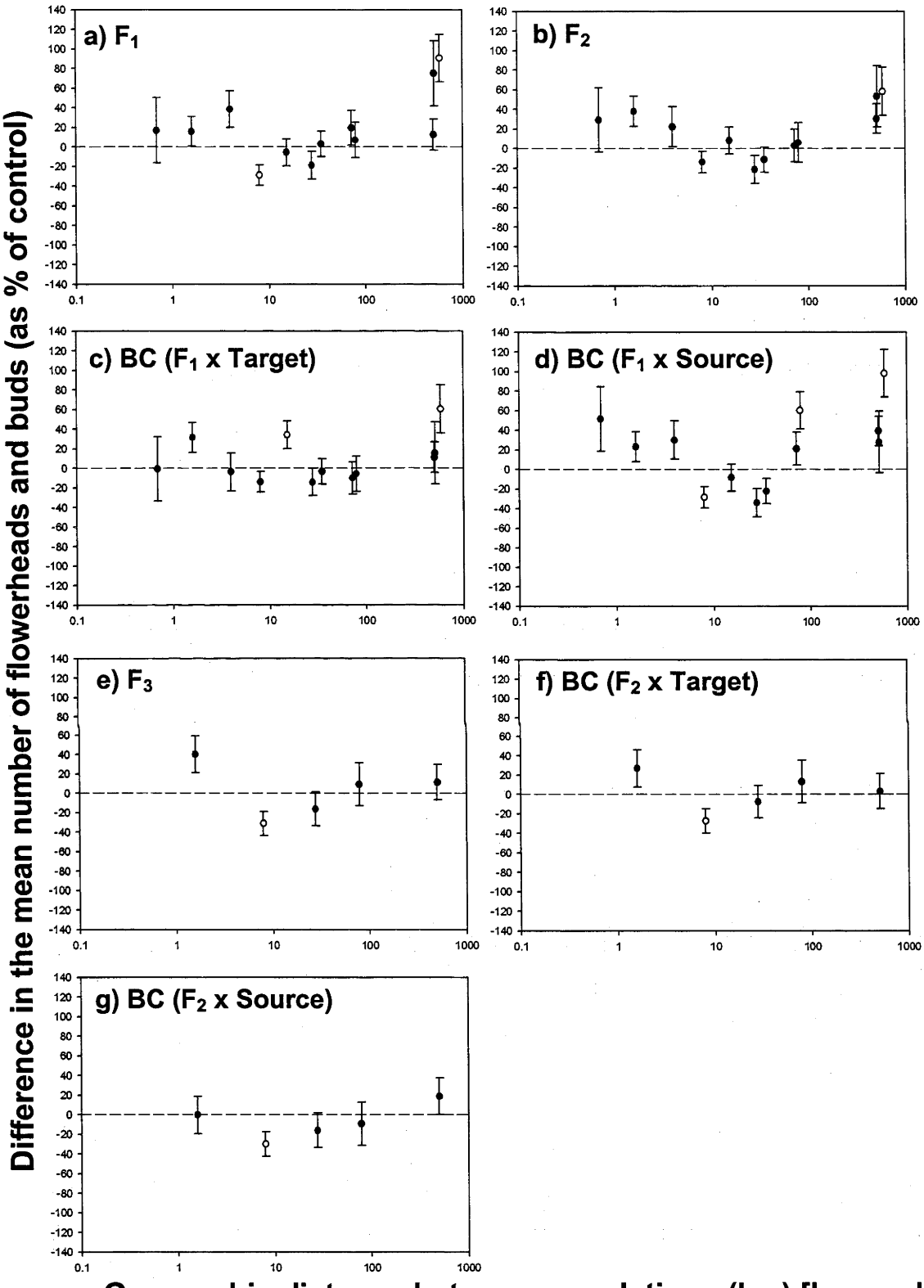

a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in mean total biomass (as % of control)
Geographic distance between populations (km) [log scale]

Figure 4.7 (on following page) The difference in the mean number of flowerheads and buds at 12 months between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.


a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in the mean number of flowerheads and buds (as % of control)
Geographic distance between populations (km) [log scale]

between 58 – 98 % was observed in all hybrid progeny within GB-TR (586.2 km, Figure 4.7 a – g; Appendix 4.4). Outbreeding depression in $NoF\&B_{12}$ was observed in only one population pair (HH-MA). For HH-MA, there was consistent outbreeding depression in 5 of the 7 crossing treatments, with a decrease in the mean $NoF\&B_{12}$ of between 27 – 31 % in the F_1 (Figure 4.7 a), $BC_{F1xSOURCE}$ (Figure 4.7 d), F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$ (Figure 4.7 e – g) generations.

4.3.6 Cumulative Fitness

Considering plant fitness across the lifecycle from germination through to adult growth and reproduction (CFI_{12}), inter-population hybridisation had no negative effects on hybrid progeny fitness for any generation in the overall analysis or within individual population pairs (Table 4.2; Figure 4.8 a – g). Instead heterosis was observed in hybrid progeny for both the overall analysis and for 5 of the 12 population pairs. For the overall analysis, there was significant heterosis in the F_2 and BC's ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) as well as for progeny in the F_3 and $BC_{F1xTARGET}$ generations (Table 4.2). Three individual population pairs showed significant heterosis in the $BC_{F1xSOURCE}$ generation, with a significant increase in CFI_{12} of 50, 55 and 65 % for $BC_{F1xSOURCE}$ progeny within GB-TR, MA-BA and MJ-GB respectively (Figure 4.8 d). The greatest increase in cumulative fitness was for F_2 progeny in GB-TR, which showed an 80 % increase in CFI_{12} compared to the control. Significant heterosis was also observed for SR-TR, with an increase in CFI_{12} of 53 % for F_2 progeny. For SR-CC, hybrid progeny in the $BC_{F1xTARGET}$, F_3 and $BC_{F2xTARGET}$ generations showed an increase in CFI_{12} of between 46 – 56 % compared to the local parental population (Figure 4.8 c, e and f).

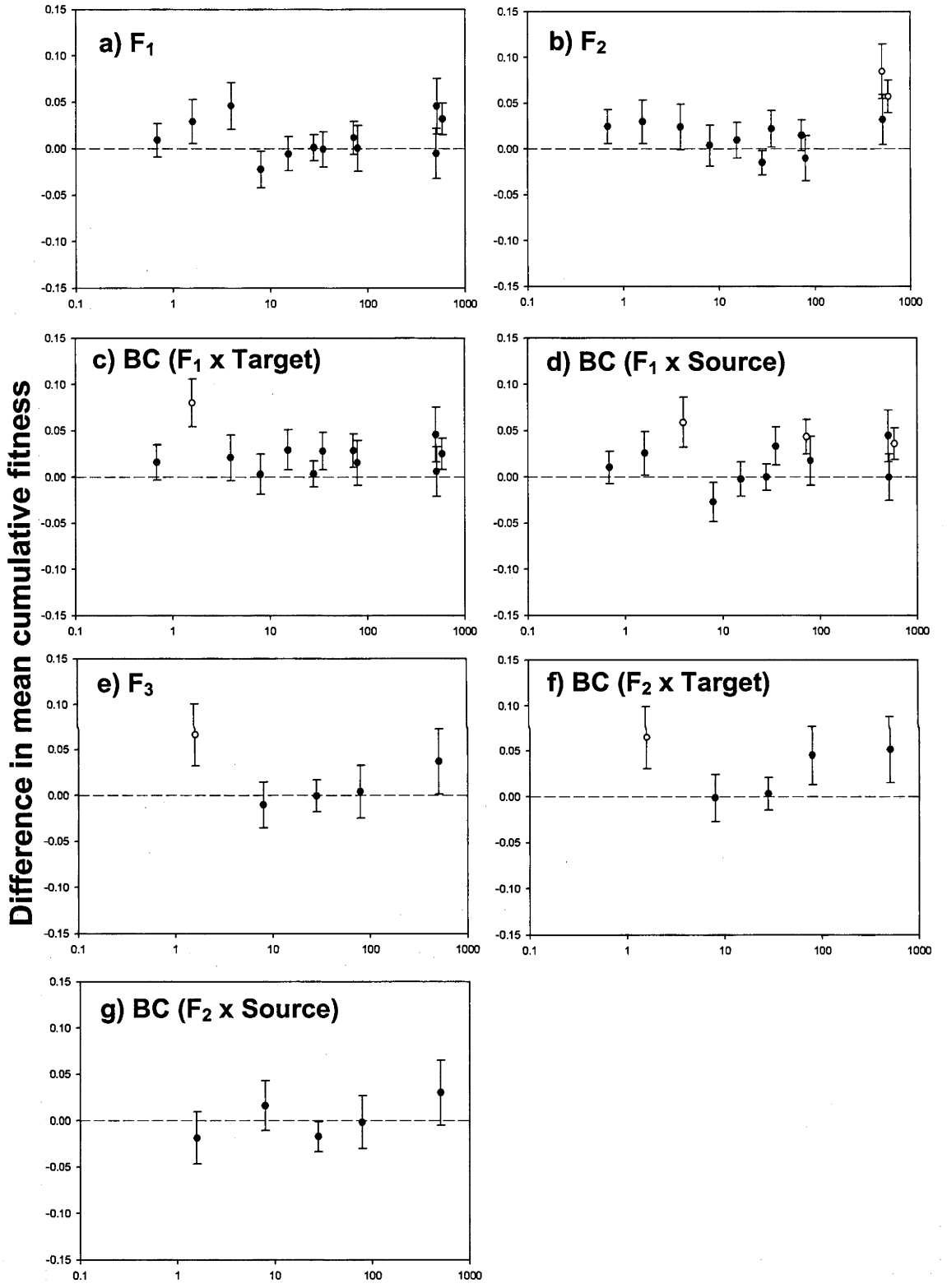
4.3.7 Predicting hybrid progeny fitness

4.3.7.1 F_1

There was a significant relationship between the difference in F_1 hybrid progeny fitness and Log Target (LogTPS) and Source (LogSPS) Population Size for a range of fitness traits across the lifecycle (Table 4.3). For the $NoLVS_8$, $NoST_8$, $NoLVS_{12}$ and $NoST_{12}$ between 32 – 42 % of the variation in F_1 hybrid progeny fitness could be explained by LogTPS and LogSPS, such that the greatest increase in F_1 fitness in these traits was for small Target and large Source populations. In addition, for F_1 progeny there was a significant positive relationship between LogSPS and HT_{12} ($R^2 = 0.28$, $P = 0.046$) and a

Figure 4.8 (on following page) The difference in mean cumulative fitness (germination *x* biomass *x* flowering stems) between the control (within Target population cross) and (a) F_1, (b) F_2, (c) BC (F_1 *x* Target), (d) BC (F_1 *x* Source), (e) F_3, (f) BC (F_2 *x* Target) and (g) BC (F_2 *x* Source) as a function of geographic distance.

Values above the dashed horizontal (zero) line represent heterosis and those below represent outbreeding depression. Population pairs where the difference between the control and crossing treatment was significantly different (○) and not significantly different (●) from zero. Vertical bars represent ± 1 standard error.


a) F1
b) F2
c) BC (F1 x Target)
d) BC (F1 x Source)
e) F3
f) BC (F2 x Target)
g) BC (F2 x Source)
Difference in mean cumulative fitness
Geographic distance between populations (km) [log scale]

marginally significant negative relationship between LogTPS and GERM ($R^2 = 0.20$, $P = 0.082$).

4.3.7.2 F_2

At the adult reproductive life stage the difference in fitness between the control and F_2 progeny was significantly related to LogTPS and LogSPS for the $NoST_{12}$ ($R^2 = 0.30$, $P = 0.08$) (Table 4.3), with the greatest fitness in small Target and large Source populations. At the adult life stage there was a marginally significant negative relationship between $INDPS_8$ and LogTPS ($R^2 = 0.25$, $P = 0.058$), so that F_2 progeny in small Target populations experienced the greatest increase in $INDPS_8$.

4.3.7.3 *BC (F_1 x Target)*

There was a significant relationship between LogTPS and LogSPS and the difference in fitness for $BC_{F1xTARGET}$ progeny compared to the control for a number of variables at the adult and reproductive adult life stages (Table 4.3). Between 42 – 64 % of the variation in $BC_{F1xTARGET}$ progeny for the $NoLVS_8$, $NoST_8$, $NoLVS_{12}$, and $NoST_{12}$ could be explained by Log Target and Log Source population size. For all these variables, the greatest increase in $BC_{F1xTARGET}$ progeny fitness was in population pairs with small Target and large Source populations. There was also a significant negative relationship between LogTPS and $INDPS_8$ ($R^2 = 0.48$, $P = 0.007$).

4.3.7.4 *BC (F_1 x Source)*

The difference in TB_{12} between the control and $BC_{F1xSOURCE}$ progeny was significantly related to Log Target and Log Source population size ($R^2 = 0.49$, $P = 0.019$), with the greatest increase in TB_{12} in pairs with small Target and large Source populations (Table 4.3).

In contrast to Log Target and Log Source population size, geographic (GeogDist) and environmental (EnvDist) distance has little power to explain the variability in the difference in hybrid progeny fitness in the F_1, F_2 and BC ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) generations.

Table 4.3 Results of the single (and multiple) linear regression analysis examining the relationship between Log Target Reproductive Population Size (LogTPS) and Log Source Reproductive Population Size (LogSPS) and hybrid progeny fitness in the F_1, F_2 and $BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$ generations for a range of fitness traits.

(–) negative relationship, (+) positive relationship. There was no significant relationship between any of the explanatory variables and hybrid progeny fitness for fitness traits and generations not included in the table.
$P < 0.01$*** highly significant; $0.01 < P < 0.05$** significant; $0.05 < P < 0.1$* marginally significant

Generation and Fitness trait	R^2	*P*	Significant explanatory variable/s	
			Slope of relationship	
			LogTPS	LogSPS
F_1				
Seedling				
Germination (GERM)	0.20	0.082*	-	
Adult (8 months)				
Number of leaves ($NoLVS_8$)	0.37	0.051*	-	+
Number of stems ($NoST_8$)	0.32	0.071*	-	+
Reproductive Adult (12 months)				
Number of leaves ($NoLVS_{12}$)	0.42	0.036**	-	+
Plant height (HT_{12})	0.28	0.046**		+
Number of stems ($NoST_{12}$)	0.40	0.017**	-	+
F_2				
Adult (8 months)				
Index of plant size ($INDPS_8$)	0.25	0.058*	-	
Reproductive Adult (12 months)				
Number of stems ($NoST_{12}$)	0.30	0.08*	-	+
$BC_{F1xTarget}$				
Adult (8 months)				
Number of leaves ($NoLVS_8$)	0.52	0.016**	-	+
Index of plant size ($INDPS_8$)	0.48	0.007***	-	
Number of stems ($NoST_8$)	0.45	0.029**	-	+
Reproductive Adult (12 months)				
Number of leaves ($NoLVS_{12}$)	0.42	0.036**	-	+
Number of stems ($NoST_{12}$)	0.64	0.004***	-	+
$BC_{F1xSource}$				
Reproductive Adult (12 months)				
Total Biomass (TB_{12})	0.49	0.019**	-	+

4.3.7.5 F_3

There was a marginally significant relationship between EnvDist and the difference in F_3 progeny fitness for $RosHT_4$ (R^2 = 0.65, P = 0.063) and $INDPS_8$ (R^2 = 0.61, P = 0.074) (Table 4.4), so that for both variables, the difference between the control and F_3 progeny increased with increasing EnvDist between populations. For the $NoST_{12}$ there was a marginally significant negative relationship with LogGeogDist, so that F_3 progeny fitness was greatest at small geographic distances. For two variables at the reproductive adult life stage ($NoLVS_{12}$ and $FLOWERST_{12}$) the difference in fitness between the control and F_3 progeny was positively related to LogTPS, while for TB_{12} the difference in F_3 progeny fitness was negatively related to LogSPS (Table 4.4). However, these relationships between progeny fitness and LogTPS and LogSPS in the F_3 (and BC generations) should be interpreted with caution due to an uneven distribution of Target and Source population sizes for population pairs with F_3 and BC progeny. For LogTPS, these relationships are primarily driven by the difference between HH-MA (TPS (HH) = 300) and the remaining 4 population pairs with large Target population sizes (TPS: 27 626 (MJ) – 95 200 (GB)), while for LogSPS they are driven by the difference between the one population pair with a large SPS (GB-PO, SPS (PO) = 8171) and the other four population pairs with small Source populations (SPS: 118 (MA) – 626 (TR)).

4.3.7.6 BC (F_2 x Target)

There was a significant positive relationship between the difference in GERM between the control and $BC_{F2xTARGET}$ progeny and EnvDist (R^2 = 0.77, P = 0.032) (Table 4.4). Geographic distance was positively related to the difference in $NoST_8$ (R^2 = 0.87, P = 0.013), with the greatest increase in $NoST_8$ for population pairs separated by larger geographic distances. For a number of fitness traits ($INDPS_8$, $NoLVS_{12}$ and $NoF\&B_{12}$) LogTPS was positively related to the difference in $BC_{F2xTARGET}$ progeny fitness compared to the control (Table 4.4). However, as discussed above, these relationships with LogTPS and LogSPS should be interpreted cautiously.

Table 4.4 Results of the single (and multiple) linear regression analysis examining the relationship between Log Target Reproductive Population Size (LogTPS), Log Source Reproductive Population Size (LogSPS), Log Geographic Distance between populations (LogGeogDist) and Environmental Distance (EnvDist) and hybrid progeny fitness for a range of fitness traits in the F_3 and BC (BC_{TARGET} (F_2 *x* Target) and BC_{SOURCE} (F_2 *x* Source)) generations.

(–) negative relationship, (+) positive relationship. There was no significant relationship between any of the explanatory variables and hybrid progeny fitness for fitness traits and generations not included in the table.
$P < 0.01$*** highly significant; $0.01 < P < 0.05$** significant; $0.05 < P < 0.1$* marginally significant
Analyses in italics should be interpreted with caution due to the uneven spread of Target and Source Reproductive Population Sizes for population pairs with progeny in the F_3 and BC generations.

Generation and Fitness trait	R^2	*P*	Significant explanatory variable/s			
			Slope of relationship			
			LogTPS	LogSPS	GeogDist	EnvDist
F_3 (F_2 x F_2)						
Juvenile (4 months)						
Rosette Height ($RosHT_4$)	0.65	0.063*				+
Adult (8 months)						
Index of plant size ($INDPS_8$)	0.61	0.074*				+
Reproductive Adult (12 months)						
Number of leaves ($NoLVS_{12}$)	*0.77*	*0.031***	+			
Number of stems ($NoST_{12}$)	0.66	0.06*			-	
Flowering Stems ($FLOWERST_{12}$)	*0.87*	*0.013***	+			
Total Biomass (TB_{12})	*0.63*	*0.069**		-		
BC (F_2 x Target)						
Seedling						
Germination (GERM)	0.77	0.032**				+
Adult (8 months)						
Index of plant size ($INDPS_8$)	*0.78*	*0.029***	+			
Number of stems ($NoST_8$)	0.87	0.013**			+	
Reproductive Adult (12 months)						
Number of leaves ($NoLVS_{12}$)	*0.82*	*0.023***	+			
Number of Flowers and Buds ($NoF\&B_{12}$)	*0.64*	*0.064**	+			
BC (F_2 x Source)						
Adult (8 months)						
Index of plant size ($INDPS_8$)	*0.83*	*0.021***	+			
Reproductive Adult (12 months)						
Number of leaves ($NoLVS_{12}$)	*0.97*	*0.001****	+			
Number of stems ($NoST_{12}$)	0.64	0.065*				-

4.3.7.7 BC (F_2 x Source)

There was a marginally significant negative relationship between EnvDist and the difference in the $NoST_{12}$ for $BC_{F2xSOURCE}$ progeny compared to the control ($R^2 = 0.64$, $P = 0.065$), with the greatest increase in hybrid progeny fitness for pairs with smaller environmental distance between populations (Table 4.4). The difference between the control and $BC_{F2xSOURCE}$ progeny for $INDPS_8$ and $NoLVS_{12}$ was significantly related to LogTPS (INDPS8: $R^2 = 0.83$, $P = 0.021$; NoLVS12: $R^2 = 0.97$, $P = 0.001$), with hybrid progeny fitness increasing with Target population size (Table 4.4). Yet as for F_3 and $BC_{F2xTARGET}$ progeny, these relationships between hybrid progeny fitness and LogTPS need to be interpreted with caution.

4.3.8 Variability in progeny fitness and population size

For a range of traits across the lifecycle from seedling germination to adult growth and reproduction there was a significant negative relationship between the variability in control and hybrid progeny fitness and LogTPS (Table 4.5). For GERM, there was a marginally significant negative relationship between the standard error of the mean and LogTPS for $BC_{F1xSOURCE}$ and $BC_{F1xTARGET}$ progeny (Table 4.5). However, for NoLVS, HT, INDPS and NoST at the adult life stage (8 months) and HT, NoST, FLOWERST and TB for the reproductive adult life stage (12 months), variation in progeny fitness was significantly related to LogTPS for all Cross Types (Table 4.5), with LogTPS explaining up to 53 % of the variation in the standard error of the mean. In contrast, for $RosHT_4$ there was a significant positive relationship between LogTPS and the standard error of the mean for each Cross Type (Table 4.5). For this trait, LogTPS explained between 39 – 49 % of the variation in the standard error of the mean across all generations (Control, F_1, F_2 and BC ($BC_{F1xSOURCE}$ and $BC_{F1xTARGET}$)).

Table 4.5 The relationship between the standard error of the means for each Cross Type (Control, F_1, F_2 and BC ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) and Log Target Reproductive Population Size (LogTPS) for a range of fitness traits across the lifecycle.

For fitness traits at each life stage not included in the table there was no relationship between the standard error of the means and LogTPS. $P < 0.01$*** highly significant; $0.01 < P < 0.05$** significant; $0.05 < P < 0.1$* marginally significant

	Cross Type														
	Control			F_1			F_2 (F_1 x F_1)			BC (F_1 x Target)			BC (F_1 x Source)		
Fitness Trait	R^2	*P*	Slope	R^2	*P*	Slope	R^2	*P*	Slope	R^2	*P*	Slope	R^2	*P*	Slope
Seedling															
Germination (GERM)	0.15	0.113		0.00	0.36		0.00	0.42		0.25	0.055*	-	0.18	0.095*	-
Juvenile (4 months)															
Rosette Height ($RosHT_4$)	0.41	0.015**	+	0.45	0.01**	+	0.46	0.009***	+	0.49	0.006***	+	0.39	0.018**	+
Adult (8 months)															
Number of leaves ($NoLVS_8$)	0.36	0.023**	-	0.28	0.045**	-	0.33	0.029**	-	0.27	0.05**	-	0.32	0.031**	-
Plant Height (HT_8)	0.42	0.014**	-	0.34	0.027**	-	0.32	0.032**	-	0.36	0.023**	-	0.38	0.02**	-
Index of plant size ($INDPS_8$)	0.53	0.005***	-	0.42	0.014**	-	0.49	0.007***	-	0.43	0.013**	-	0.46	0.01**	-
Number of stems ($NoST_8$)	0.39	0.017**	-	0.30	0.038**	-	0.33	0.031**	-	0.32	0.034**	-	0.43	0.013**	-
Reproductive Adult (12 months)															
Plant height (HT_{12})	0.31	0.034**	-	0.23	0.067*	-	0.18	0.094*	-	0.21	0.074*	-	0.23	0.064*	-
Number of stems ($NoST_{12}$)	0.36	0.023**	-	0.26	0.051*	-	0.26	0.052*	-	0.26	0.05**	-	0.38	0.019**	-
Flowering Stems ($FLOWERST_{12}$)	0.38	0.019**	-	0.33	0.031**	-	0.31	0.035**	-	0.26	0.053*	-	0.39	0.018**	-
Total Biomass (TB_{12})	0.37	0.022**	-	0.26	0.053*	-	0.30	0.039**	-	0.28	0.046**	-	0.31	0.035**	-

4.4 Discussion

An examination of the fitness effects of inter-population hybridisation in *R. leptorrhynchoides* resulted in five key findings that have important implications for understanding the potential risks of outbreeding depression and the evolutionary process of population divergence. These findings include:

i. For the overall analysis across all population pairs, and for the majority of individual population pair comparisons, the fitness of inter-population hybrids was either equal to or greater than the local parental population for a range of fitness traits across the life-cycle.

ii. Hybrid progeny fitness varied across generations, between different fitness traits and among different population pairs. Across generations, heterosis was greatest in the F_2 and BC's ($BC_{F1xTARGET}$ and $BC_{F1xSOURCE}$) for a range of fitness traits. For traits identified through elasticity analysis as having the greatest demographic importance, including seedling survivorship, total biomass (as a surrogate for adult survivorship) and reproduction, there was no evidence of outbreeding depression in the overall analysis or for 10 of the 12 individual population pairs. Significant heterosis was observed in the F_2, F_3 and BC generations for seedling survival, as well as for F_2 and BC progeny in some pairs for total biomass and reproduction. SR-CC and HH-MA were the only two population pairs in which hybrid progeny were less fit than the local parental population.

iii. Assessing fitness across the lifecycle from germination through to reproduction (cumulative fitness), inter-population hybrids had either equal or greater fitness than the local parental population.

iv. Log reproductive Target and Source population size had the greatest power to predict the fitness outcomes of inter-population hybridisation, while geographic and environmental distance had little power to explain the variation in hybrid progeny fitness, and

v. The variability in fitness of control and hybrid progeny was greatest in small populations.

4.4.1 Variation in hybrid progeny fitness across generations

For the majority of studies of inter-population hybridisation, heterosis (hybrid vigour) has generally been found to dominate the fitness response in F_1 progeny (Busch 2006; Byers 1998; Fenster & Galloway 2000a; Luijten et al. 2002; Sheridan & Karowe 2000; van Treuren et al. 1993; see also Table 1.1) either as a result of overdominance or the masking of deleterious recessive alleles (Lynch & Walsh 1998). However, like the present study where F_1 progeny fitness was either equal to or less than the local parental population (except for $RosHT_4$), others studies have found limited heterosis (e.g. Bailey & McCauley 2006; Keller et al. 2000; Paland & Schmid 2003) or outbreeding depression (e.g. Eckstein & Otte 2005; Fischer & Matthies 1997; Montalvo & Ellstrand 2001) in F_1 hybrids. Yet despite the lack of heterosis in most fitness traits, the negative relationship between Target population size and F_1 fitness suggests that small populations of *R. leptorrhynchoides* are bi-parentally inbred and experience a greater increase in fitness in the F_1 compared to large populations. Moreover, the fitness of hybrid progeny is determined by the interaction of a range of genetic processes (see Figure 1.1) so that the loss of local adaptation, underdominance and the disruption of additive *x* additive epistasis may counteract heterosis in the F_1, resulting in no overall increase in fitness (Lynch 1991). With the relative importance of each of these processes dependent on the degree of population differentiation (Galloway & Etterson 2005; Lynch 1991).

For *R. leptorrhynchoides*, heterosis was greatest in F_2 and BC progeny for a range of fitness traits. This is in contrast to other studies where F_2 fitness is generally equal to or less than the parental populations (Erickson & Fenster 2006; Fenster & Galloway 2000b; Keller et al. 2000), although for *C. fasciculata* an increase in F_2 fitness was also observed for some traits (Erickson & Fenster 2006; Fenster & Galloway 2000b). This increase in fitness in the F_2 compared to F_1 generation, especially for germination, juvenile growth and cumulative fitness indicates that heterosis may carry through to the F_2 and, in the absence of hybrid breakdown, lead to increased fitness in recombinant hybrid progeny. In addition, the input of genetic variation through hybridisation and the potential for recombination to produce advantageous gene combinations (Erickson & Fenster 2006) may enhance fitness in the F_2 and BC generations, although recombination may equally produce disadvantageous gene combinations. The

importance of Target population size in predicting the fitness outcomes for F_2 and BC progeny indicates that heterosis is transmitted to subsequent generations, which results in hybrid vigour at various levels of admixture. In addition, the restoration of parental genotypes through segregation may result in some recovery from the loss of additive *x* additive epistasis and underdominance in the F_2 and BC's, which may contribute to increased fitness in these generations. Local adaptation and the loss of coadaptation following recombination may also be important in determining the fitness outcomes of inter-population hybrids. The relative roles of these two genetic mechanisms will be discussed in detail in chapter 5.

For the majority of fitness traits and population pairs there was no difference in the fitness of F_3 and BC progeny compared to the local parental population. However, for some traits (e.g. germination and seedling survivorship) the fitness of inter-population hybrids was greater than the local population. Outbreeding depression was only expressed consistently in one population pair (HH-MA) and for this pair reduced fitness in hybrid progeny was not found exclusively in the F_3, but was constant across generations. In comparison, hybrid breakdown for a range of fitness traits was delayed until the F_3 for *C. fasciculata* (Fenster & Galloway 2000a, b). However, like the current study, fitness was often equal to and sometimes greater than parental populations, suggesting that in both studies heterosis may offset any decrease in fitness through the loss of coadaptation (Fenster & Galloway 2000a). Furthermore, the combination of increased genetic variation in hybrid progeny and recombination in the F_3 may promote fitness through the generation of novel gene combinations (Erickson & Fenster 2006).

4.4.2 Variation in hybrid progeny fitness across the lifecycle

For *R. leptorrhynchoides* variability in the fitness consequences of outbreeding was observed both across the life cycle and between different fitness traits at each life stage. Across all population pairs heterosis was most consistent in seedling and juvenile traits, especially in the F_2 and BC generations. This is in contrast to the results of Galloway and Etterson (2005) where, for *Campanula americana*, greater outbreeding depression was expressed in juvenile compared to later life traits. Variation in the expression of inbreeding depression across the life cycle could explain some of the variability in heterosis between the different life stages. If there was increased inbreeding depression

in seedling and juvenile traits, then greater heterosis following inter-population hybridisation may be expected at these life-stages. Heterosis may then carry through to subsequent generations, leading to increased progeny fitness in the F_2 and BC generations for seedling and juvenile traits, especially with recovery from the loss of additive *x* additive epistasis and underdominance in later generations. However, for outcrossing species, greater inbreeding depression has been observed at the seed production and adult growth/reproduction life stages, rather than for germination and juvenile survival (Husband & Schemske 1996). Furthermore, Target population size had little power to predict hybrid progeny fitness for juvenile traits and was only significantly related to germination in the F_1, which suggests that for juvenile traits heterosis was not greatest in small (bi-parentally inbred) populations. Yet given high spatial structure (Wells & Young 2002) and dominance between *S*-alleles in sporophytic self-incompatibility systems (Schierup et al. (1998); see also chapter 6) bi-parental inbreeding may occur in both large and small populations of *R. leptorrhynchoides*. Therefore the variability in heterosis across the lifecycle could be due to a combination of the differential expression of inbreeding depression (Husband & Schemske 1996) and differences in the genetic basis of population differentiation in juvenile compared to adult traits (Galloway & Etterson 2005).

4.4.3 Demographically important traits and cumulative fitness

Variation in the fitness effects of inter-population hybridisation across the lifecycle is important for long-term population viability if there are differences in the expression of heterosis and/or outbreeding depression between traits of greatest demographic importance at each life stage. Across all population pairs, inter-population hybridisation had no negative effects on progeny fitness for demographically important traits (see Young et al. 2000b) including seedling survival, total biomass (as a surrogate for adult survival) and reproduction. In comparison, heterosis for these traits was greatest in the F_2 and BC generations. As for inbreeding depression (as shown by Ouborg & Van Treuren 1997), this has important implications for predicting the long-term fitness outcomes of outbreeding and suggests that for *R. leptorrhynchoides*, inter-population hybridisation would have either no effect or in some cases could potentially benefit long-term viability. Similarly, for cumulative fitness, hybrid progeny fitness was either equivalent to or greater than the local parental population, indicating that for this species

fitness benefits may accumulate through the lifecycle, even when outcrossing over large spatial scales of up to 600 km. Interestingly, for HH-MA (8.0 km) and SR-CC (1.5 km) which were the two population pairs with consistent evidence of outbreeding depression across different fitness traits, cumulative fitness across different generations was either equal to (HH-MA and SR-CC) or greater than (SR-CC) the local parental population, suggesting that outbreeding depression was not consistent and that heterosis may counteract outbreeding depression over the lifecycle for these population pairs.

4.4.4 Predicting the fitness consequences of inter-population hybridisation

Log Target and Source population size had the greatest power to predict hybrid progeny fitness in the F_1, F_2 and backcross generations, with the greatest increase in hybrid progeny fitness observed in small Target and large Source populations. Increased fitness in hybrid progeny from small Target populations most likely reflects genetic load and bi-parental inbreeding in small populations of *R. leptorrhynchoides*, despite previous research suggesting low bi-parental inbreeding in this species (Young et al. 2000b). The relationship between Source population size and hybrid progeny fitness was unexpected and may be due to a number of potential mechanisms. Firstly, a general positive relationship between population size and heterozygosity (e.g. Leimu et al. 2006) means that sourcing from large populations with greater levels of heterozygosity may have beneficial effects for hybrid progeny fitness. For *R. leptorrhynchoides*, however, heterozygosity was unrelated to population size (Young et al. 1999). Secondly, greater genetic diversity in large populations (see Young et al. 2000b; Young et al. 1999) may provide more variation for the evolution of novel, highly fit genotypes through recombination (Erickson & Fenster 2006). Although this could potentially contribute to the increased fitness of progeny in recombinant generations (F_2 and BC), it is unlikely to drive the relationship between Source population size and F_1 fitness. An alternative explanation is that genetic drift in small populations may result in population differentiation for additive *x* additive epistasis. Consequently, for small Source populations, inter-population hybridisation will disrupt these favourable combinations of alleles leading to reduced hybrid progeny fitness. Greater fitness in hybrid progeny from large Source populations may reflect the reduced potential of genetic drift to generate additive x additive epistasis in large populations, as the relative importance of

drift to genetic architecture and population differentiation decreases with increasing population size (Barrett & Kohn 1991; Sherwin & Moritz 2000).

In contrast to population size, geographic and environmental distance between populations had very little power to predict hybrid progeny fitness in the F_1, F_2 and BC generations. The only trend for a relationship with spatial scale was for the number of leaves at the juvenile life stage ($NoLVS_4$) where the greatest increase in hybrid progeny fitness in the F_2 and BC generations was at intermediate (10 – 100 km) distances (see Figure 4.3). This lack of relationship between fitness and spatial scale is similar to the results of Fenster and Galloway (2000b) and Montalvo and Ellstrand (2001) where geographic distance had no consistent effect on hybrid fitness, even over large spatial scales. In comparison, the degree of outbreeding depression in F_1 progeny was related to population proximity for *C. americana* (Galloway & Etterson 2005) while for *Lotus scoparius* F_1 fitness decreased with increasing environmental distance between parental populations (Montalvo & Ellstrand 2001). The predictive power of geographic and environmental distance will depend on the relative importance of selection, drift, gene flow and spatial patterns of environmental heterogeneity in determining population differentiation (Edmands 2002). This suggests that for *R. leptorrhynchoides,* rather than spatial scale or environmental heterogeneity, it is small population processes which are fundamental in determining the evolutionary trajectory of populations and the degree of population differentiation.

For the F_3 and BC generations, however, geographic and environmental distance were more important in explaining hybrid progeny fitness, although the predictive power of these matrices was often inconsistent and varied between fitness traits and generations. For example, for the $NoST_{12}$ for $BC_{F2xSOURCE}$ progeny, hybrid fitness decreased with increasing environmental distance, while for GERM for BC progeny ($BC_{F2xTARGET}$) and $RosHT_4$ and $INDPS_8$ in the F_3, hybrid fitness increased with environmental distance between populations. The relationship between hybrid fitness and geographic distance was also contradictory, with the greatest increase in $NoST_{12}$ for F_3 hybrids at smaller spatial scales, while for $NoST_8$, $BC_{F2xTARGET}$ hybrid progeny fitness was greatest at large geographic distances. However, given the small sample size (n = 5 populations) in the F_3 and BC generations and the inherent variation between population pairs, these

(often weak) relationships maybe simply be sampling artefacts. On the other hand, the importance of environmental distance and spatial scale may only become apparent in later generations as the initial benefits of heterosis (driven by population size) decline in subsequent generations. As highlighted in section 4.3.7, the relationship between hybrid fitness and Target and Source population size should be treated with caution due to the uneven spread of Target and Source population sizes in the F_3 and BC generations. In this case, the positive relationship between progeny fitness and LogTPS for a number of variables was driven by consistent outbreeding depression in HH-MA, which was also the only population pair in the F_3 and BC generations with a small Target population size.

4.4.5 Variability in hybrid progeny fitness and population size

An increase in the importance of small population processes (genetic drift and inbreeding) as population size declines can result in reduced fitness in small populations due to loss of genetic diversity and an increase in the expression of deleterious alleles through inbreeding (Young et al. 1996). This relationship between population size and mean fitness has been demonstrated empirically in a number of studies (for reviews see Keller & Waller 2002; Leimu et al. 2006). However, in addition to reductions in mean fitness, these processes may also increase inter-plant variability in fitness, as observed in the current study. In this case, progeny fitness was negatively related to population size such that small populations had the greatest variation in fitness for a range of traits across the lifecycle. Increased drift and potential for bi-parental inbreeding together with high spatial structure (Wells & Young 2002) and a reduction in effective population size with the loss of *S*-alleles in small populations (Young et al. 2000b; see chapter 6) can result in large differences in progeny fitness between maternal families within small populations. This increased variability in small parental populations then translates to greater variation in hybrid progeny fitness over successive generations. In addition, selection should reduce the variability for life-history traits with major impact on fitness (high elasticity) (de Kroon et al. 2000; Picó et al. 2003) so that increased variation in traits with high elasticity values in small populations of *R. leptorrhynchoides* (i.e. total biomass (TB_{12}) and flowering stems ($FLOWERST_{12}$)) reflects the greater significance of drift rather than selection in determining genetic structure in small populations. Therefore, taken together, both the increase in variability

in progeny fitness in small populations and the importance of population size in predicting the fitness outcomes of inter-population hybridisation suggest that small population processes play a fundamental role in determining hybrid fitness, patterns of genetic variation and population differentiation in this species.

CHAPTER 5:
GENETIC MECHANISMS UNDERLYING OUTBREEDING DEPRESSION IN FRAGMENTED POPULATIONS OF *Rutidosis leptorrhynchoides*

5.1 Introduction

Outbreeding depression and the fitness consequences of inter-population hybridisation are difficult to predict and can vary considerably across species (e.g. Keller et al. 2000) as well as between populations, environments and among different fitness traits within a single species (e.g. Dudash & Fenster 2000; Fenster & Galloway 2000b). This variability in the expression of outbreeding depression is due to complexities in the underlying genetic mechanisms and the interaction of these different mechanisms with the evolutionary forces of selection, genetic drift, gene flow and mutation.

Outbreeding depression is primarily determined by two genetic mechanisms that are not mutually exclusive (Lynch 1991), but may act independently or synergistically to determine the fitness outcomes of inter-population hybridisation. These two mechanisms are: (1) the loss of local adaptation due to the mixing of gene pools adapted to different environmental selection pressures (Cena et al. 2006; Dudash & Fenster 2000; Montalvo & Ellstrand 2001) and; (2) the disruption of co-adapted gene complexes (or intrinsic co-adaptation) which represent favourable epistatic gene interactions that have evolved in different populations (Falconer & Mackay 1996; Fenster & Galloway 2000a; Lynch 1991). However, a number of other genetic mechanisms including additive *x* additive epistasis, cytoplasmic *x* nuclear epistasis, underdominace, maternal boosting and heterosis (see section 1.3.3) may contribute to determining the fitness outcomes of inter-population hybridisation. Consequently, both additive and non-additive modes of gene action are likely to contribute to the expression of outbreeding depression and determination of the fitness of hybrid progeny.

Understanding the relative contribution of additive and non-additive genetic variation to population differentiation and divergence is important in the study of outbreeding depression for three reasons; Firstly, it may assist in predicting patterns of outbreeding depression and the fitness consequences of inter-population hybridisation. Secondly, it can provide important insights into the mechanisms by which micro-evolutionary processes within a population, driven by genetic drift, selection, mutation and gene flow, translate to the macro-evolutionary process of population divergence, reproductive isolation and speciation (Lynch & Walsh 1998). Finally, an examination of non-additive effects following inter-population hybridisation can provide evidence for the relative importance of epistasis in the evolutionary process (Fenster et al. 1997; Wade 2002), which to date has only been tested empirically in a limited number of studies (e.g. Fenster & Galloway 2000b; Galloway & Etterson 2005).

The role and importance of epistasis as a mechanism of evolutionary change has long been a topic of debate, and is fundamental to the different models of evolution proposed by Fisher and Wright (reviewed in Wade & Goodnight 1998). However, epistatic population differentiation can play an important role in the response of a population to selection (Wade & Goodnight 1998) and for many theories of speciation is a central component of the transition from micro- to macro-evolution (Burke et al. 1998; Dobzhansky 1937; Muller 1942, (Dobzhansky-Muller model); Rieseberg 1997; Wade 2002). Indeed, theories underlying both outbreeding depression and speciation are based on the epistatic interaction of genes within the specific genetic backgrounds of different populations (Wade 2002). It is therefore important to determine the relative contribution of both local adaptation and epistatic interactions to population differentiation and the genetic architecture of adaptive evolution.

There are a number of factors that may affect population divergence and through this outbreeding depression including mating system, gene flow, effective population size, selection regimes, drift and bi-parental inbreeding (see section 1.3.5). These factors can interact with the different genetic mechanisms underlying outbreeding depression and hence examining the relative importance of these genetic mechanisms can provide an understanding of the evolutionary processes and factors that influence population divergence. Following this, the various measures or surrogates of population divergence

may vary in their power to predict outbreeding depression, depending on the underlying genetic mechanism. For example, environmental distance as a proxy of adaptive population differentiation may correlate with outbreeding depression based on the dilution of genes associated with local adaptation. In contrast, geographic distance between populations may have more predictive power when outbreeding depression is based on disruption of adaptive epistasis.

A number of recent studies have provided important contributions to our understanding of the genetic basis of outbreeding depression and population divergence, and have included assessments of epistasis and the breakdown of co-adapted gene complexes (Bailey & McCauley 2006; Fenster & Galloway 2000b; Keller et al. 2000), cyto-nuclear epistasis (Galloway & Etterson 2005; Galloway & Fenster 1999; Pelabon et al. 2005) and local adaptation (Fenster & Galloway 2000a, b; Galloway & Fenster 2000; Montalvo & Ellstrand 2000, 2001). However, despite these studies, there is still limited knowledge of the relative contribution of the different mechanisms to the expression of outbreeding depression and to date no studies have concurrently assessed the importance of these two mechanisms empirically, particularly over a number of generations.

As discussed in section 1.3 and illustrated in Figure 1.1, the fitness outcomes of inter-population hybridisation may vary across generations due to the differential expression, and interaction of a number of potential genetic mechanisms. Examining the relative contribution of these different mechanisms will assist in addressing the question of whether heterosis is able to offset hybrid breakdown as well as explaining the overall fitness outcomes of inter-population hybridisation across multiple generations. Moreover, selection will reduce both additive and epistatic genetic variance for traits closely correlated with fitness (i.e. life history traits) compared to morphological traits (Roff & Emerson 2006). Therefore, the contribution of additive and non-additive genetic variation to population differentiation may vary between different traits, depending on their correlation with fitness, which can be defined in a broad sense by elasticity values from demographic analysis. Consequently, examining the role of additive and epistatic gene action in population differentiation for different traits across

the lifecycle can provide insights into both the process of selection and the interaction of selection with different modes of gene action.

Given that both local adaptation and the disruption of co-adapted gene complexes may operate synergistically to influence the fitness of hybrid progeny, partitioning the effects of these two mechanisms empirically involves controlling for one mechanism, while altering the other. The aim of this chapter is to use this methodology to examine the relative contribution of these two different genetic mechanisms to the expression of outbreeding depression, and assess their relationship to population divergence and adaptive differentiation by addressing the following questions:

1. Local adaptation: Controlling for the level of recombination, does diluting the average proportion of local genes influence the fitness of hybrid progeny?
2. Co-adapted gene complexes: At a certain level of admixture, does the degree of recombination influence the fitness of hybrid progeny?
3. Do these two genetic mechanisms act in concert to influence the fitness of hybrid progeny?
4. Does the relative importance of each genetic mechanism vary between fitness traits across the lifecycle?
5. Does cytoplasmic *x* nuclear epistatsis contribute to progeny fitness?
6. Can geographic and environmental distance between parental populations explain the relative importance of each genetic mechanism?

5.2 Materials and methods

5.2.1 Crossing design and pollination experiment

The crossing design and methodology reported in this chapter is fully described in Chapter 4 (section 4.2). To partition the influence of each potential genetic mechanism, this experiment required the creation of backcrossed progeny to the Target and Source populations in both the second and third generations (outlined in Figure 5.1). For all 12 population pairs this gave three levels of admixture for the crosses with one round of recombination (i.e. the F_2 (F_1 *x* F_1) and backcrosses ($BC_{F1xTARGET}$ and $BC_{F1x\ SOURCE}$) see Figure 5.1), and the three levels of admixture for the 5 population pairs with F_3 (F_2 *x* F_2) and backcrossed progeny ($BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$) (Figure 5.1) which had

undergone a subsequent round of recombination. Co-adapted gene complexes were assessed using progeny with a set level of admixture (50 % local genes) in each generation. This design enabled the influence of local adaptation (through admixture analysis) and the breakdown of co-adapted gene complexes (through analysis of the effect of recombination) on progeny fitness to be assessed both independently and concurrently.

As described above, an examination of local adaptation involved separate admixture analysis for the second (including all 12 population pairs) and third (5 population pairs) generations. Hence this enabled the effect of admixture to be assessed at two levels of recombination. Consequently, the results of the admixture analysis in the two generations will be presented separately. Furthermore, an examination of the interaction of admixture and recombination was assessed using the 5 population pairs with F_2, F_3 and BC progeny for both generations (see Figure 5.1)

5.2.2 Growth experiment and monitoring

Planting of the growth experiment and monitoring of progeny fitness for the experiments reported in this chapter are outlined in section 4.2, as they were included as part of the outbreeding depression experiment as described in chapter 4.

5.2.3 Statistical analysis

5.2.3.1 Local adaptation (admixture analysis) and Co-adapted gene complexes (recombination analysis)

Generalised Linear Models (GERM and CFI_{12}) and REML Linear Mixed Models ($NoLVS_{4,8}$, $RosHT_4$, HT_8, TB_{12} and $NoF\&B_{12}$) were used to assess the influence of admixture level (in the second and third generations) and recombination on progeny fitness for a range of fitness traits across the life-cycle by examining differences between Cross Types as described in section 4.2.7 (4.2.7.4 GLM – logistic regression; 4.2.7.5 GLM – General model; 4.2.7.6 REML Linear Mixed Models). For each statistical model (for both the overall analysis including all population pairs and for each independent population pair comparison) if the global test was significant ($P < 0.05$) then LSD's were used to assess statistical differences between admixture levels in each generation. For the recombination analysis, LSD's were used to assess differences

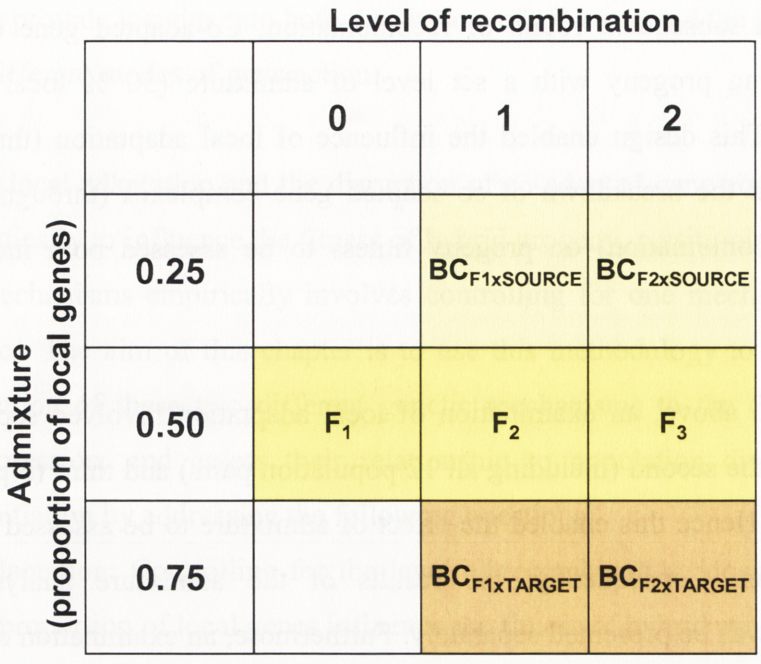


Figure 5.1 Crossing structure to partition the contribution of each genetic mechanism to progeny fitness.

Horizontal rows represent the three levels of admixture (0.25, 0.5 and 0.75 local genes), while the vertical columns indicate the level of recombination. In the F_1 recombination is zero (0), with the first round of recombination (1) in the second generation while the third generation has undergone a second round of recombination (2).

between progeny with different levels of recombination but a set level of admixture (i.e. F_1, F_2 and F_3 progeny).

5.2.3.2 Interaction between admixture and recombination

Generalised Linear Models (GERM and CFI_{12}) and REML Linear Mixed Models ($NoLVS_{4,8}$, $RosHT_4$, HT_8, TB_{12} and $NoF\&B_{12}$) were used to assess if there was an interaction between admixture and recombination, suggesting that these two mechanisms may act in concert to influence hybrid progeny fitness. Only F_2, $BC_{F1xTARGET}$, $BC_{F1xSOURCE}$, F_3, $BC_{F2xTARGET}$ and $BC_{F2xSOURCE}$ Cross Types for the five population pairs with second and third generation progeny were included in this analysis. For both the GLM and REML models seed weight was fitted as a covariate in the overall analysis and for each individual population pair (except MJ-CF). For the GLM's seed weight, Maternal Line *x* Block and Admixture *x* Recombination (which expands to Admixture + Recombination + Admixture *x* Recombination) were fitted

sequentially in the model. For the analysis across all population pairs, Maternal Line was nested within Population Pair. Accumulated Analysis of Deviance was used to assess the significance of the model and each term individually. For the REML's Admixture *x* Recombination (Admixture + Recombination + Admixture *x* Recombination) was fitted as the main effect in the fixed model, while Maternal Line *x* Block was fitted in the random model. For the analysis across all population pairs, Maternal Line was nested within Population Pair in the random model.

5.2.3.3 Cytoplasmic-nuclear epistasis

The effect of cytoplasmic background on F_1 hybrid progeny fitness was assessed using Generalised Linear Models (GERM and CFI_{12}) and REML Linear Mixed Models ($NoLVS_{4,8}$, $RosHT_4$, HT_8, TB_{12} and $NoF\&B_{12}$). This analysis focussed on F_1 hybrid progeny because from the reciprocal crosses in each population pair approximately half of the maternal lines in the F_1 were randomly chosen to have a local cytoplasmic background (i.e. the maternal plant originated from the Target population) while the remaining half had a foreign cytoplasmic background (i.e. the maternal plant came from the Source population) (see section 4.2.2.2 for details). For the GLM Block, Maternal Line and Cytoplasmic Background were fitted sequentially into the model. For the REML's, Cytoplasmic Background was fitted as the main effect in the fixed model, while Block and Maternal Line (nested within Cytoplasmic Background) were fitted in the random model. For the overall analysis Maternal Line was nested within Population Pair in the random model.

5.3 Results

5.3.1 Local adaptation

5.3.1.1 Seedling

5.3.1.1.1 Germination (GERM)

For the overall analysis including all population pairs (Table 5.1) and for 9 of the 12 individual population pairs (Figure 5.2) there was no significant difference in mean germination between the different levels of admixture in the second generation. However, there was a significant decline in germination with a decrease in the

proportion of local genes (i.e. evidence of local adaptation) for 3 population pairs representing a range of geographic distances (SR-CC (1.5 km) Figure 5.2 b; MJ-CF (27.8 km) Figure 5.2 f; GB-PO (78.9 km) Figure 5.2 i). For SR-CC and GB-PO the greatest decrease in germination (13 and 31 % respectively) was between 75 % ($BC_{F1xTARGET}$) and 50 % (F_2) local genes, while for MJ-CF a 12 % decrease in germination was observed between 75 % and 25 % local genes.

In contrast, for the overall analysis including all population pairs in the third generation there was a significant decrease in germination between 75 % and 25 % local genes (Table 5.1). However, for the individual population pairs, only SR-CC (Figure 5.3 a) showed a significant decease in germination of 23 % between 50 % (F_3) and 25 % ($BC_{F2xSOURCE}$) admixture, while there were no significant differences in germination at different levels of admixture for the remaining 4 population pairs (Figure 5.3 b – e).

5.3.1.2 Juvenile (4 months)

5.3.1.2.1 Number of leaves ($NoLVS_4$)

In the second generation, admixture level had no significant influence on the $NoLVS_4$ across all population pairs (Table 5.1) and for 10 of the 12 individual population pair comparisons (Figure 5.4 a, b, d – k). There was a significant increase in the mean $NoLVS_4$ between 75 % and 25 % admixture for MA-BA (4.0 km; Figure 5.4 c) and between 75 % and 50 % admixture for GB-TR (586.2 km; Figure 5.4 l), suggesting that for these two population pairs increasing the proportion of foreign genes resulted in a significant increase in the mean $NoLVS_4$.

Admixture level had a significant influence on $NoLVS_4$ for the overall analysis in the third generation (Table 5.1), but not for any of the individual population pair comparisons (Figure 5.5 a – e). Across all population pairs there was a significant increase in the mean $NoLVS_4$ between 75 % and 50 % admixture, but no significant difference between 25 % local genes and the other levels of admixture indicating that the greatest increase in NoLVS was in the F_3 (50 % admixture) (Table 5.1).

Table 5.1 The results of the overall admixture analysis including all population pairs of *Rutidosis leptorrhynchoides* for the second (n = 12) and third (n = 5) generations for a range of fitness traits across the lifecycle.

Wald statistics and *P* values represent the results of the global statistical model testing for significant differences between all crossing treatments. Comparisons between admixture levels in the second and third generations represent subsequent LSD comparisons for each generation. For each fitness trait and analysis different letters indicate statistically significant differences between crossing treatments ($P < 0.05$). Values in parentheses represent one standard error. [1]Deviance ratio for GERM and CFI_{12}

			Second generation			Third generation		
			Level of admixture (% local genes)			Level of admixture (% local genes)		
Fitness trait	Wald/d.f.[1]	*P* value	$BC_{F1xTARGET}$ (75 %)	F_2 (50 %)	$BC_{F1xSOURCE}$ (25 %)	$BC_{F2xTARGET}$ (75 %)	F_3 (50 %)	$BC_{F2xSOURCE}$ (25 %)
Seedling								
Germination (GERM) (%)	9.38	<0.001	78.2 (1.3) [a]	76.0 (1.3) [a]	75.2 (1.3) [a]	87.7 (2.4) [a]	85.5 (2.5) [ab]	80.2 (2.8) [b]
Juvenile								
Number of leaves ($NoLVS_4$)	6.59	<0.001	13.3 (0.3) [a]	13.8 (0.3) [a]	13.4 (0.3) [a]	12.0 (0.6) [a]	14.1 (0.6) [b]	12.6 (0.6) [ab]
Rosette Height ($RosHT_4$) (mm)	7.05	<0.001	29.8 (0.4) [a]	30.8 (0.4) [a]	30.3 (0.4) [a]	28.9 (0.9) [a]	28.3 (0.9) [a]	27.3 (1.0) [a]
Adult								
Number of leaves ($NoLVS_8$)	1.83	0.076	103.8 (1.6) [a]	107.4 (1.6) [a]	104.7 (1.7) [a]	101.5 (3.6) [a]	103.0 (3.6) [a]	107.3 (3.7) [a]
Plant Height (HT_8) (mm)	3.94	<0.001	189.5 (2.4) [a]	190.2 (2.5) [a]	188.2 (2.5) [a]	179.9 (5.4) [a]	174.6 (5.3) [a]	180.8 (5.4) [a]
Reproductive adult								
Total biomass (TB_{12}) (g)	1.71	0.101	2.65 (0.03) [a]	2.71 (0.03) [a]	2.58 (0.03) [a]	2.62 (0.07) [a]	2.61 (0.07) [a]	2.57 (0.07) [a]
Number of flowers and buds ($NoF\&B_{12}$)	1.52	0.155	2.25 (0.08) [a]	2.37 (0.08) [a]	2.37 (0.08) [a]	2.12 (0.16) [a]	2.18 (0.16) [a]	2.01 (0.17) [a]
Cumulative fitness index	3.82	<0.001	0.130 (0.01) [a]	0.129 (0.01) [a]	0.126 (0.01) [a]	0.138 (0.01) [a]	0.127 (0.01) [ab]	0.113 (0.01) [b]

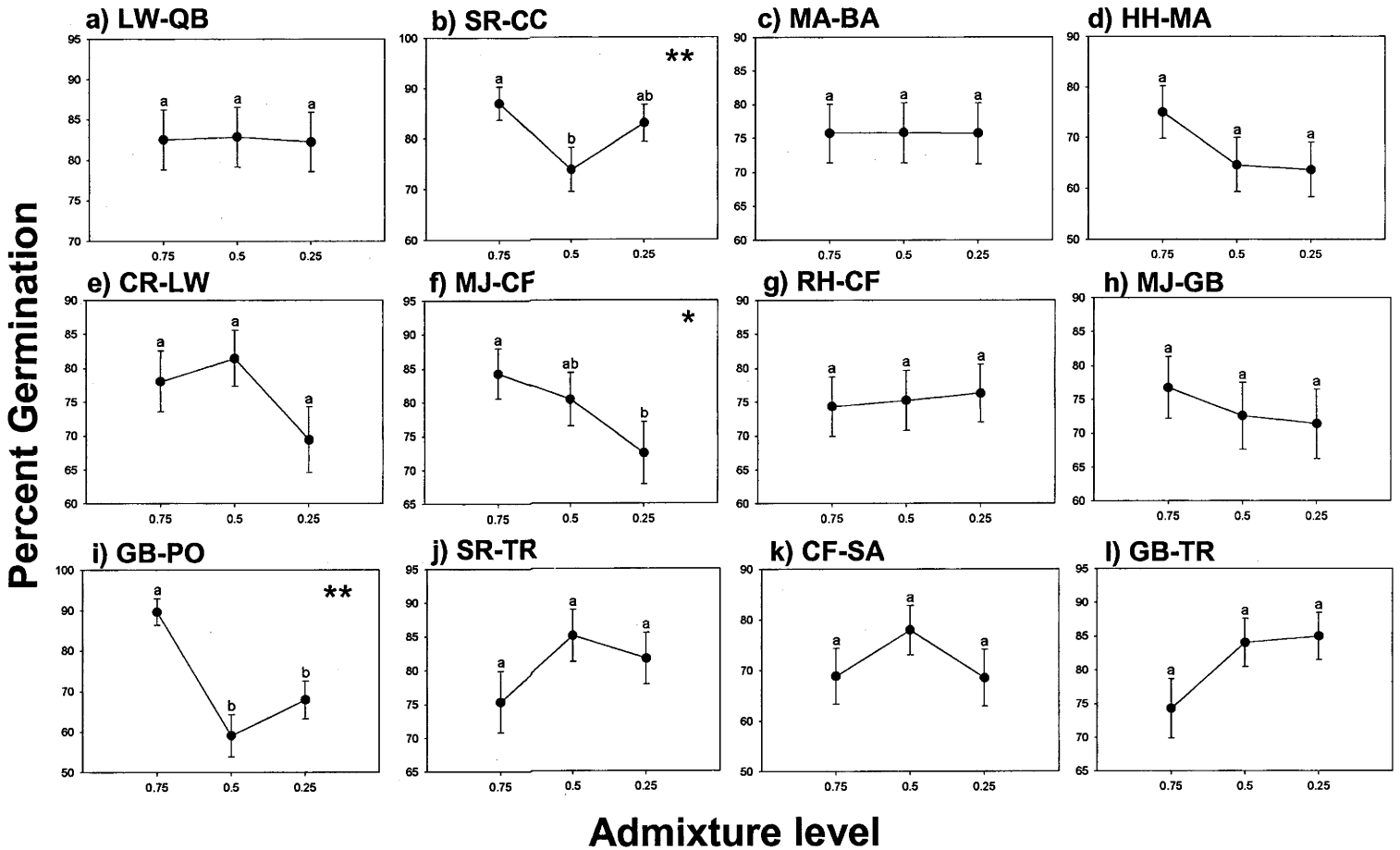


Figure 5.2 The difference in percent germination between the three levels of admixture ($BC_{F1xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in the second generation for 12 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

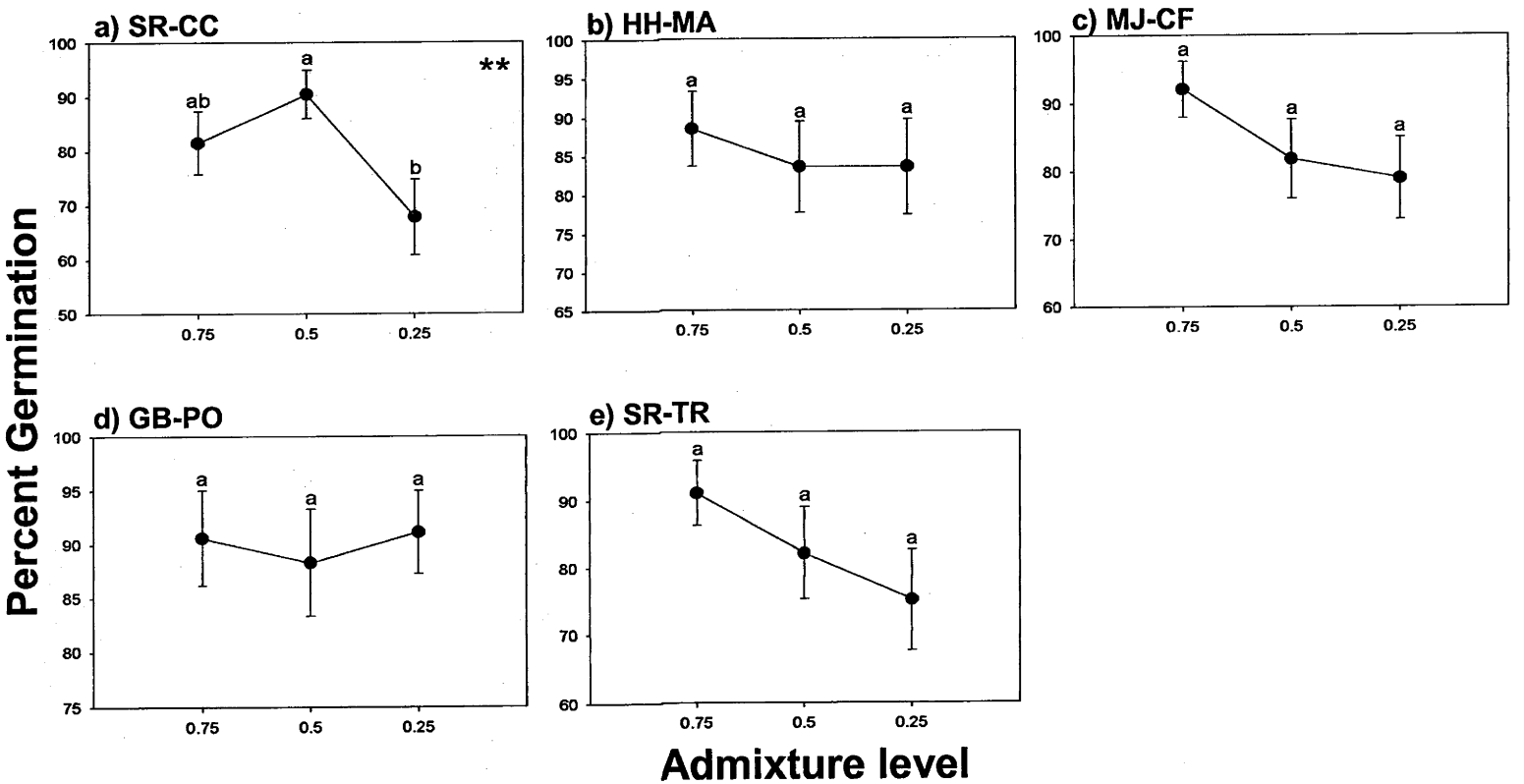


Figure 5.3 The difference in percent germination between the three levels of admixture ($BC_{F2xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F2x\ SOURCE}$ (25 % local genes)) in the third generation for 5 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

5.3.1.2.2 Rosette height ($RosHT_4$)

For the overall analysis in the second generation (Table 5.1) and for the majority (8 of 12) population pairs (Figure 5.4 a, c – g, i, j) there was no significant difference in $RosHT_4$ between the different levels of admixture. For MJ-GB (71.9; Figure 5.4 h), CF-SA (516.0 km; Figure 5.4 k) and GB-TR (586.2 km; Figure 5.4 l) there was a significant increase in $RosHT_4$ between 75 % ($BC_{F1xTARGET}$), 50 % (F_2) and 25 % ($BC_{F1xSOURCE}$) admixture suggesting that increasing the proportion of foreign genes resulted in a significant increase in mean $RosHT_4$. SR-CC (1.5 km; Figure 5.4 b) was the only population pair which showed evidence of local adaptation with a significant decrease in $RosHT_4$ between 50 % (F_2) and 25 % ($BC_{F1xSOURCE}$) admixture.

For the third generation, admixture level had no significant influence on $RosHT_4$ for the overall analysis (Table 5.1) or in any of the 5 individual population pairs (Figure 5.5 a – e).

5.3.1.3 Adult (8 months)

5.3.1.3.1 Number of leaves ($NoLVS_8$)

For the overall analysis including all population pairs (Table 5.1) and for half (6) of the individual population pairs (Figure 5.6 a, c, h – k) admixture level had no significant effect on the mean $NoLVS_8$ in the second generation. For two population pairs (SR-CC (1.5 km) and GB-TR (586.2 km)) the mean $NoLVS_8$ increased with a decline in the proportion of local genes, with a significant increase in the $NoLVS_8$ between 75 % ($BC_{F1xTARGET}$) and 50 % (F_2) admixture for SR-CC (Figure 5.6 b) and between 75 % ($BC_{F1xTARGET}$) and 25 % ($BC_{F1xSOURCE}$) for GB-TR (Figure 5.6 l). There was evidence of local adaptation in four population pairs with a significant decrease in the mean $NoLVS_8$ between 75 % ($BC_{F1xTARGET}$) and 50 % (F_2) admixture for CR-LW (15.2 km; Figure 5.6 e) and between 50 % (F_2) and 25 % ($BC_{F1xSOURCE}$) admixture for HH-MA (8.0 km; Figure 5.6 d), MJ-CF (27.8 km; Figure 5.6 f) and RH-CF (34.8 km; Figure 5.6 g)).

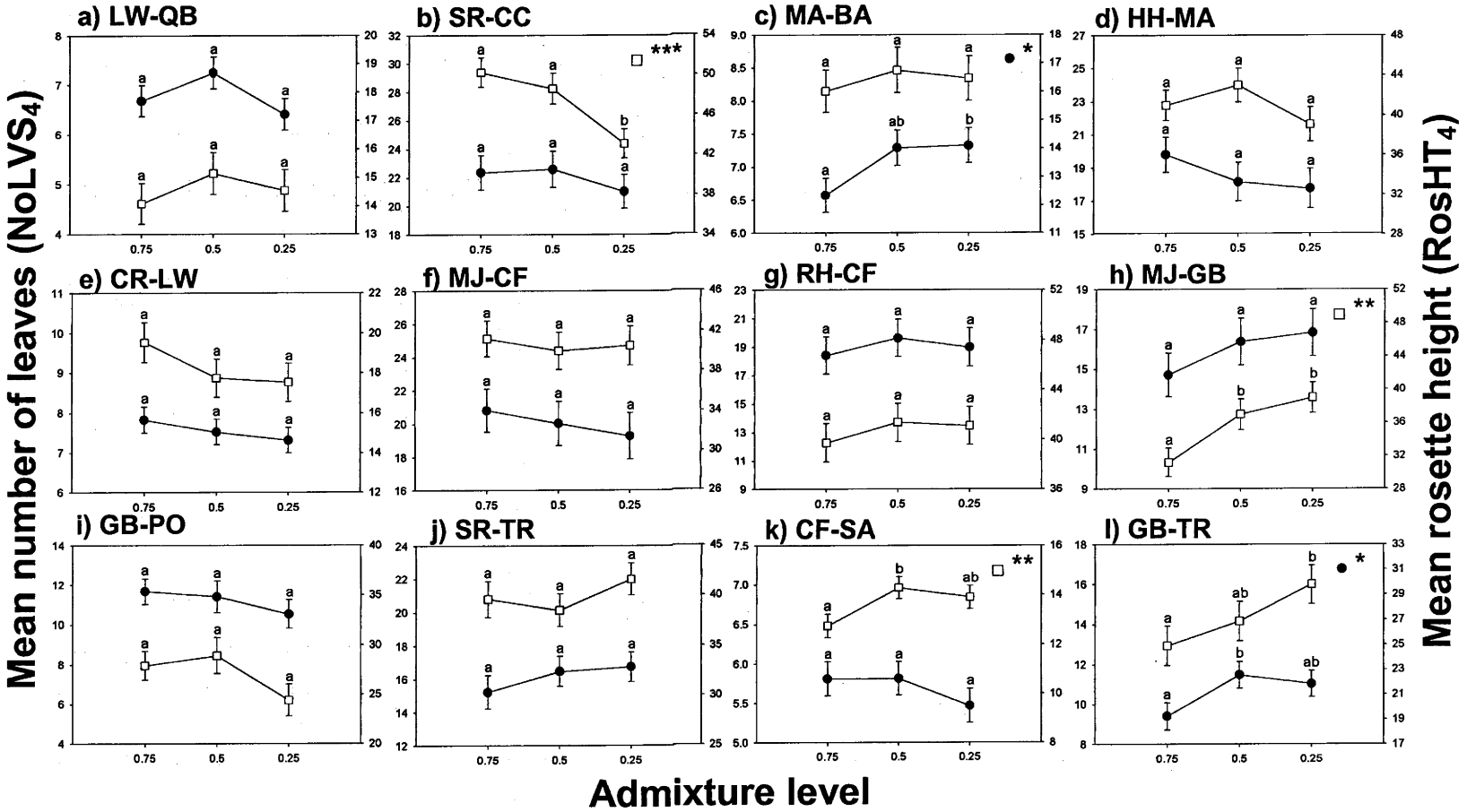


Figure 5.4 The difference in the mean number of leaves ($NoLVS_4$) (•) and mean rosette height ($RosHT_4$) (□) between the three levels of admixture ($BC_{F1xTARGET}$(75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in the second generation for the juvenile life stage in 12 population pairs of *Rutidosis leptorrhynchoides.*

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

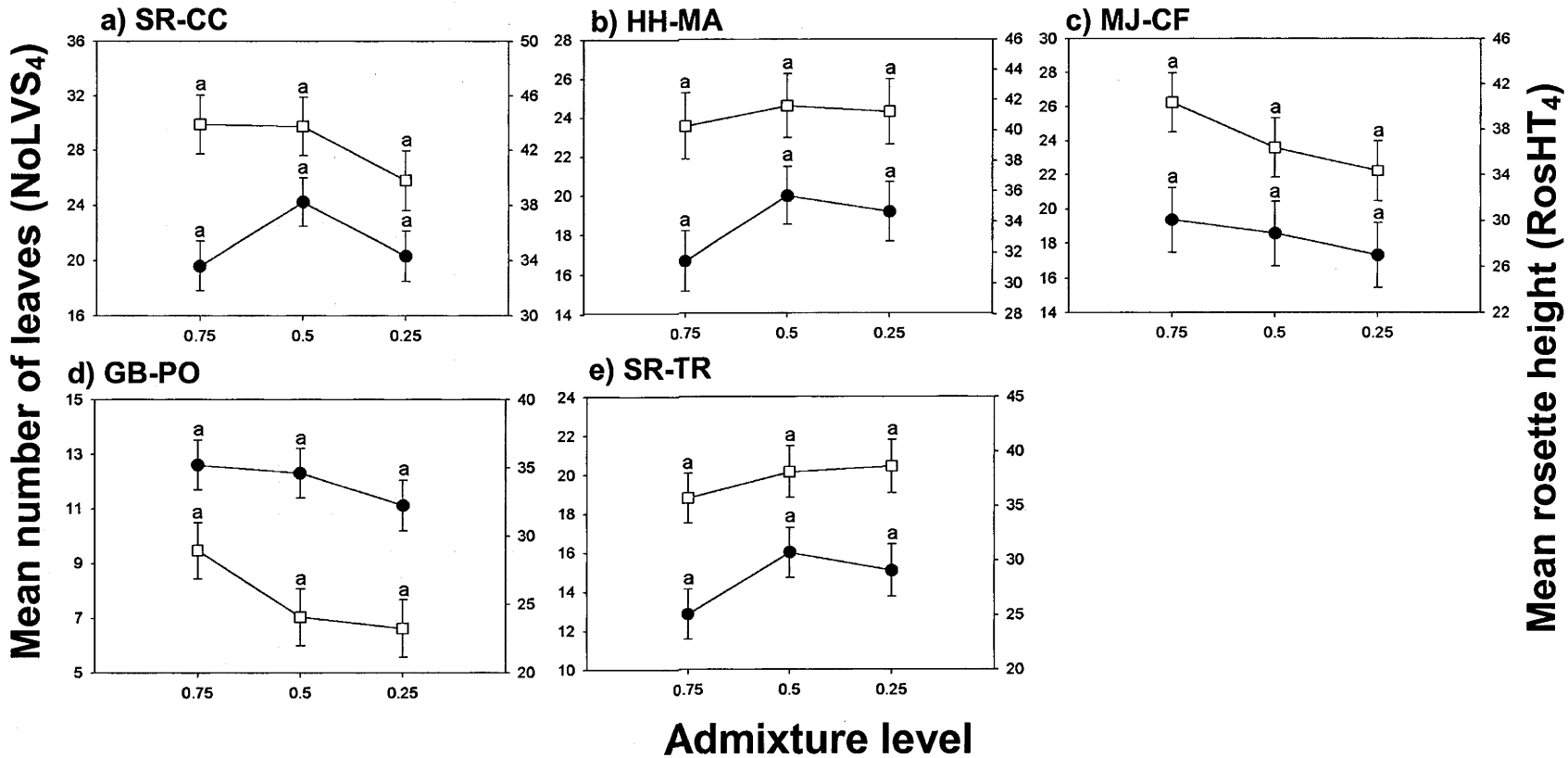


Figure 5.5 The difference in the mean number of leaves ($NoLVS_4$) (•) and mean rosette height ($RosHT_4$) (□) between the three levels of admixture ($BC_{F1xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in the third generation for the juvenile life stage in 5 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

For the third generation, admixture level had no significant effect on the mean $NoLVS_8$ for the overall analysis including all population pairs (Table 5.1) or for any of the individual population pair comparisons (Figure 5.7 a – e).

5.3.1.3.2 Plant height (HT_8)

Admixture level had no significant effect on mean HT_8 for the overall analysis (Table 5.1) and within 8 of the 12 population pairs (Figure 5.6 a, c, e, g – i, k, l). There was evidence of local adaptation in three population pairs (SR-CC (1.5 km; Figure 5.6 b), HH-MA (8.0 km; Figure 5.6 d) and SR-TR (506.2 km; Figure 5.6 j) with significant declines in HT_8 between 75 % ($BC_{F1xTARGET}$) and 50 % (F_2) admixture for SR-CC and between 75 % ($BC_{F1xTARGET}$) and 25 % ($BC_{F1xSOURCE}$) for HH-MA. For SR-TR there was a consistent decline in mean HT_8 as the proportion of local genes decreased, with significant differences between all three admixture levels (Figure 5.6 j). For MJ-CF (27.8 km) mean HT_8 increased as the proportion of local genes declined, with a significant increase in HT_8 between 50 % and 25 % admixture (Figure 5.6 f) in this population pair.

In the third generation, there was no significant difference in the mean $NoLVS_8$ between the three admixture levels for the overall analysis including all population pairs (Table 5.1) or for any of the individual population pair comparisons (Figure 5.7 a – e).

5.3.1.4 Reproductive adult (12 months)

5.3.1.4.1 Total biomass (TB_{12})

Admixture level had no significant influence on TB_{12} for the overall analysis (Table 5.1) and for 9 of the 12 population pairs (Figure 5.8 a – c, e – g, i – k) in the second generation. For MJ-GB (71.9 km) there was a significant increase in TB_{12} between 75 % and 25 % admixture (Figure 5.8 h), while for GB-TR (586.2 km) TB_{12} was significantly higher at 50 % admixture compared to 75 % and 25 % local genes (Figure 5.8 l). HH-MA was the only population pair with evidence of local adaptation, with a significant decrease in TB_{12} between 50 % (F_2) and 25 % ($BC_{F1xSOURCE}$) admixture (Figure 5.8 d).

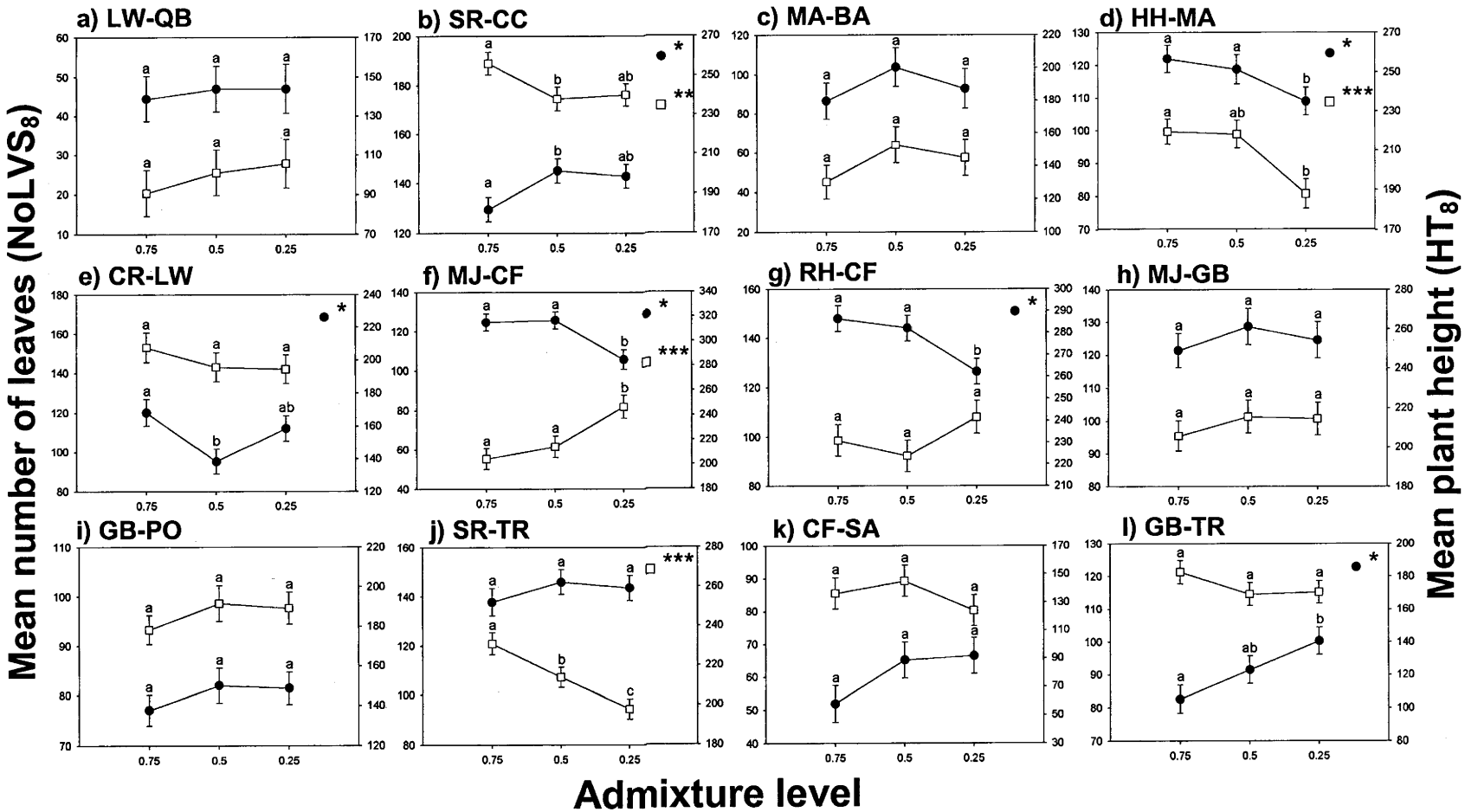


Figure 5.6 The difference between the three levels of admixture ($BC_{F1xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in the mean number of leaves ($NoLVS_8$) (•) and mean plant height (HT_8) (□) in the second generation for the adult life stage in 12 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error.
* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

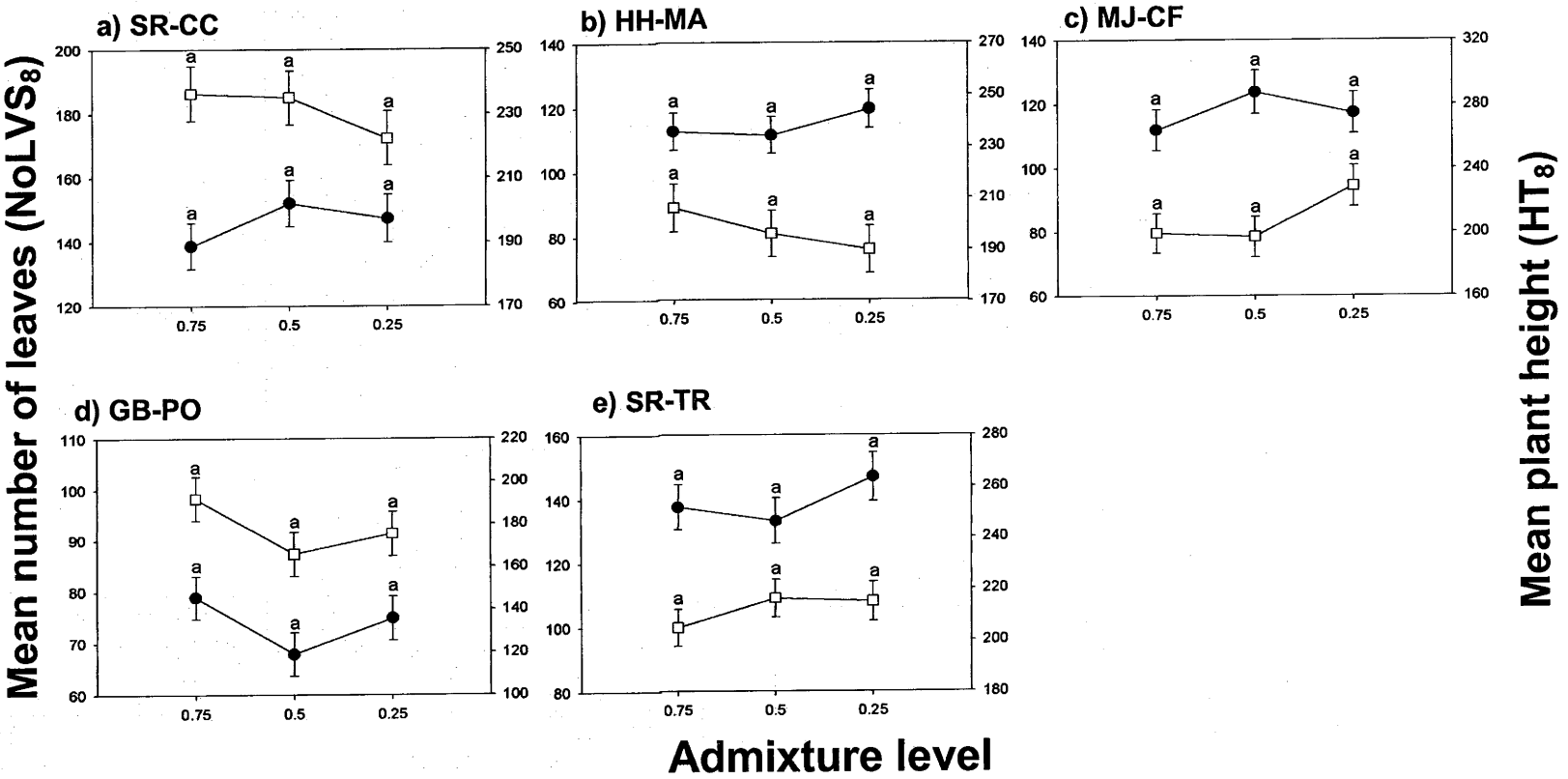


Figure 5.7 The difference between the three levels of admixture ($BC_{F1xTarget}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ Source}$ (25% local genes)) in the mean number of leaves ($NoLVS_8$) (•) and mean plant height (HT_8) (□) in the third generation for the juvenile life stage in 5 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

In the third generation, admixture level had no significant effect on TB_{12} for the overall analysis across all population pairs (Table 5.1) or for 4 of the 5 individual population pair comparisons (Figure 5.9 a – c, e). GB-PO was the only population pair with significant differences between the three levels of admixture, with a significant decrease in TB_{12} at 50 % (F_3) compared to 75 % ($BC_{F1xTARGET}$) and 25 % ($BC_{F1xSOURCE}$) admixture (Figure 5.9 d).

5.3.1.4.2 Number of flowerheads and buds ($NoF\&B_{12}$)

For the overall analysis across all population pairs (Table 5.1) and for 10 of the 12 population pairs (Figure 5.8 a – d, f – h, j – l) admixture level had no significant influence on the $NoF\&B_{12}$ in the second generation. For GB-PO (78.9 km) there was a significant increase in $NoF\&B_{12}$ between the F_2 (50 %) and $BC_{F1xSOURCE}$ (25 %) (Figure 5.8 i), with the greatest $NoF\&B_{12}$ in the admixture level with the highest proportion of foreign genes. CR-LW was the only population pair with evidence of local adaptation with a significant decrease in the $NoF\&B_{12}$ between 75 % ($BC_{F1xTARGET}$) and 25 % ($BC_{F1xSOURCE}$) local genes (Figure 5.8 e).

There was no significant effect of admixture on the $NoF\&B_{12}$ in the third generation for the overall analysis across all population pairs (Table 5.1) or for any of the 5 individual population pair comparisons (Figure 5.9 a – e).

5.3.1.5 Cumulative fitness (CFI_{12})

Admixture level had no significant effect on CFI in the overall analysis (Table 5.1) and for 11 of the 12 individual population pairs (Figure 5.10 a, c – l) in the second generation. SR-CC (1.5 km) was the only population pair with a significant difference between admixture levels, with a decrease in CFI between 75 % ($BC_{F1xTARGET}$) and 25 % ($BC_{F1xSOURCE}$) local genes (Figure 5.10 b).

In the third generation, admixture level had a significant effect on CFI for the overall analysis (Table 5.1) and for 1 of the 5 population pairs (SR-CC (1.5 km); Figure 5.10 b). There was evidence of local adaptation in the overall analysis including all population pairs, with a significant decrease in CFI between 75 % ($BC_{F2xTARGET}$) and 25 % ($BC_{F2xSOURCE}$) admixture (Table 5.1). For SR-CC, there was a significant decline in

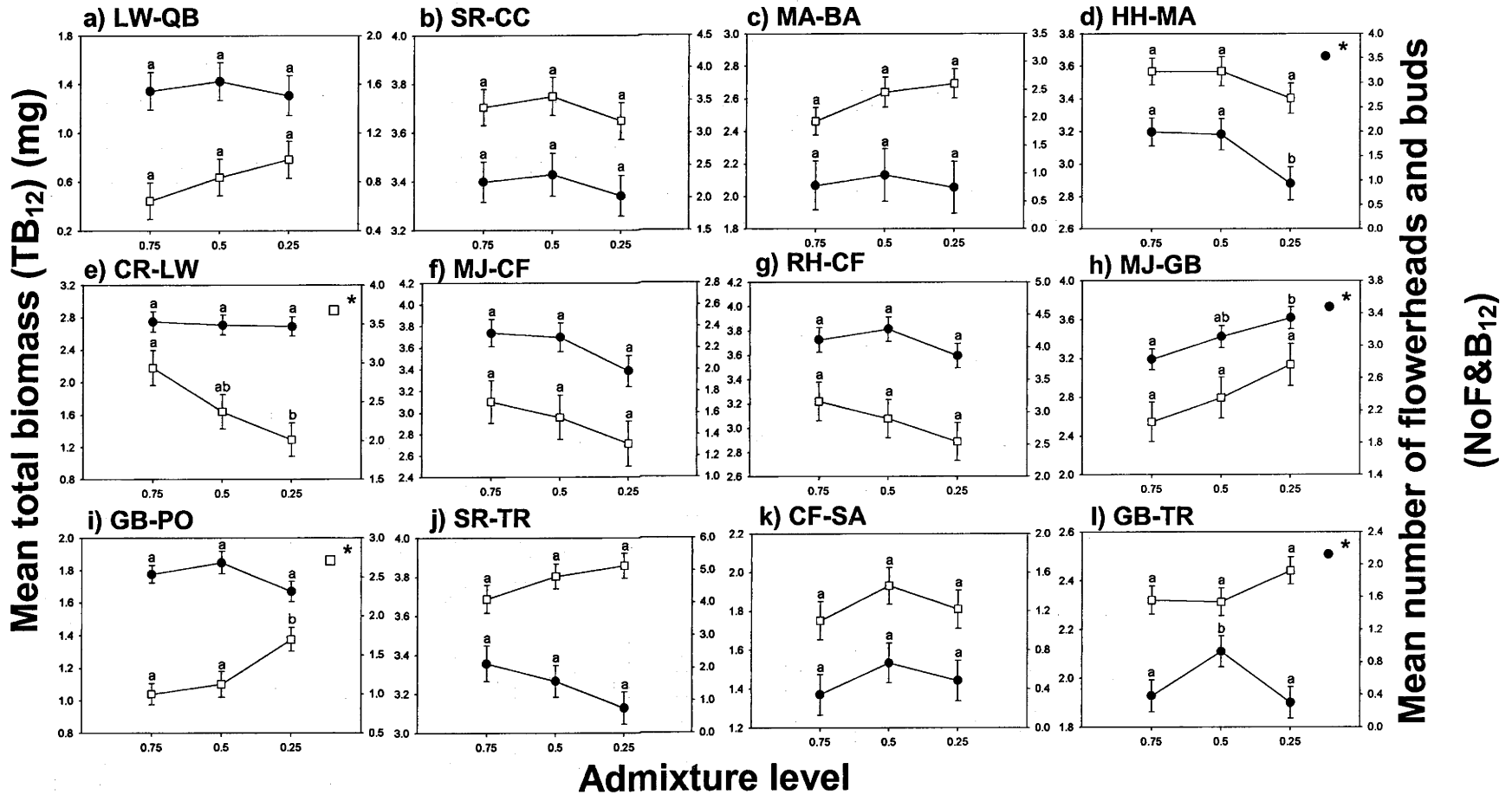


Figure 5.8 The difference between the three levels of admixture ($BC_{F1xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in mean total biomass (TB_{12}) (•) and the mean number of flowerheads and buds ($NoF\&B_{12}$) (□) in the second generation for the reproductive adult life stage in 12 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error.
* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

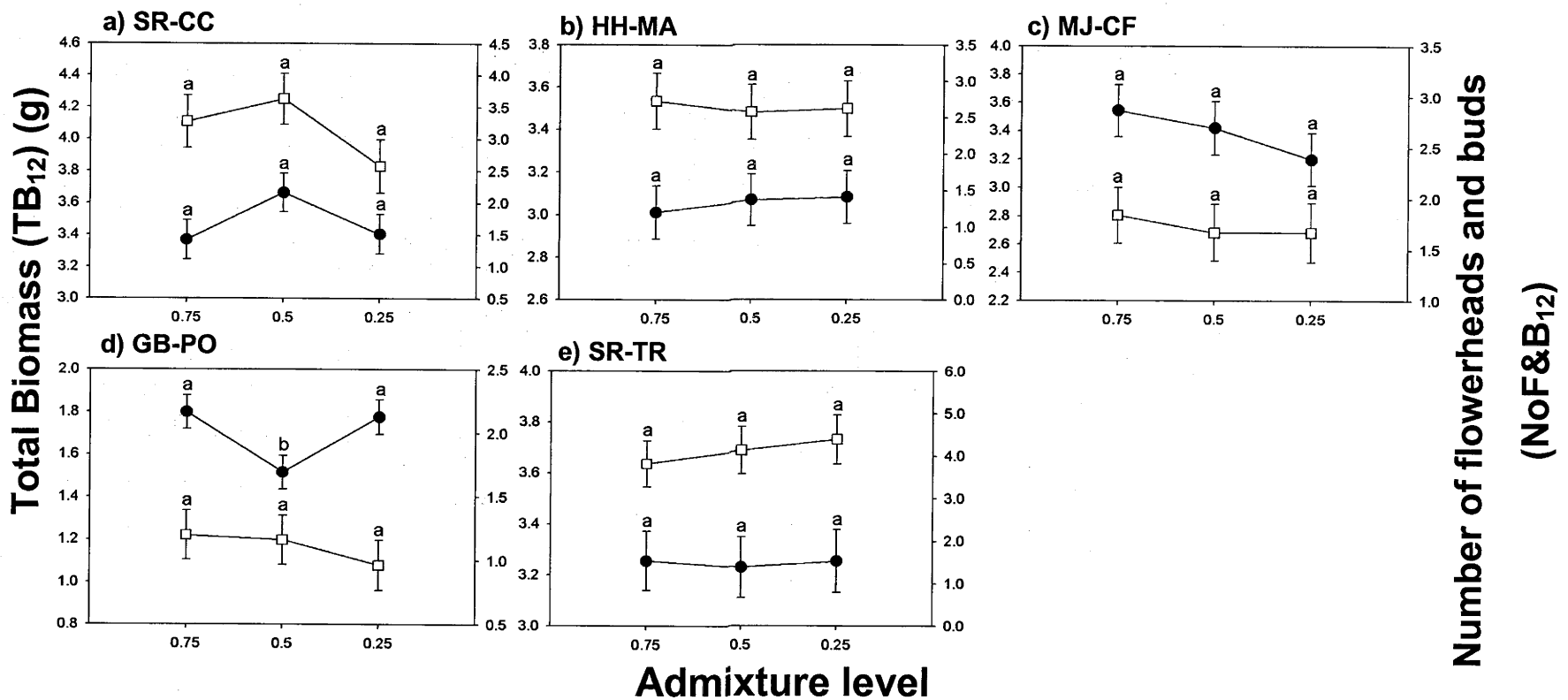


Figure 5.9 The difference between the three levels of admixture ($BC_{F2xTARGET}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F2x\ SOURCE}$ (25% local genes)) in mean total biomass (TB_{12}) (•) and the mean number of flowerheads and buds ($NoF\&B_{12}$) (□) in the third generation for the reproductive adult life stage in12 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error.
* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

CFI between 50 % (F_3) and 25 % ($BC_{F2xSOURCE}$) admixture, but no difference in CFI between 75 % ($BC_{F2xTARGET}$) and 50 % (F_3) local genes (Figure 5.11 a).

5.3.2 Recombination

5.3.2.1 Seedling

5.3.2.1.1 Germination (GERM)

There was a significant effect of recombination level on GERM for the overall analysis including all population pairs ($P < 0.001$; Table 5.2) and for 6 of the 12 individual population pairs (Figure 5.12 b, d, g, i – k). For the overall analysis there was a consistent increase in GERM between the F_1, F_2 and F_3 generations, suggesting that increased recombination had a beneficial effect on GERM (Table 5.2). For RH-CF (34.8 km; Figure 5.12 g), SR-TR (506.2 km; Figure 5.12 j) and CF-SA (516.0 km; Figure 5.12 k) GERM increased significantly between the F_1 and F_2, while for SR-CC (1.5 km; Figure 5.12 b) and HH-MA (8.0 km; Figure 5.12 d) there was no significant difference in GERM between the F_1 and F_2, but GERM did significantly increase between the F_2 and F_3 generations. For GB-PO there was a significant decrease in germination between the F_1 and F_2, but in the F_3 GERM increased above the value for both the F_1 and F_2 generations (Figure 5.12 i).

5.3.2.2 Juvenile (4 months)

5.3.2.2.1 Number of leaves ($NoLVS_4$)

Increasing recombination across generations had a significant effect on the mean $NoLVS_4$ in the overall analysis including all population pairs ($P < 0.001$; Table 5.2) and for 3 of the 12 individual population pairs (Figure 5.13 b, g, l). For the overall analysis there was a significant increase in mean $NoLVS_4$ between the F_1 and F_2 generations, but no further increase between the F_2 and F_3 (Table 5.2). For RH-CF (34.8 km; Figure 5.13 g) and GB-TR (586.2 km; Figure 5.12 l) the mean $NoLVS_4$ increased significantly between the F_1 and F_2 generations. For SR-CC (1.5 km; Figure 5.13 b), with F_1, F_2 and F_3 progeny, there was a significant increase in the mean $NoLVS_4$ between the F_1 and F_2, but no difference in the $NoLVS_4$ between the F_2 and F_3 generations.

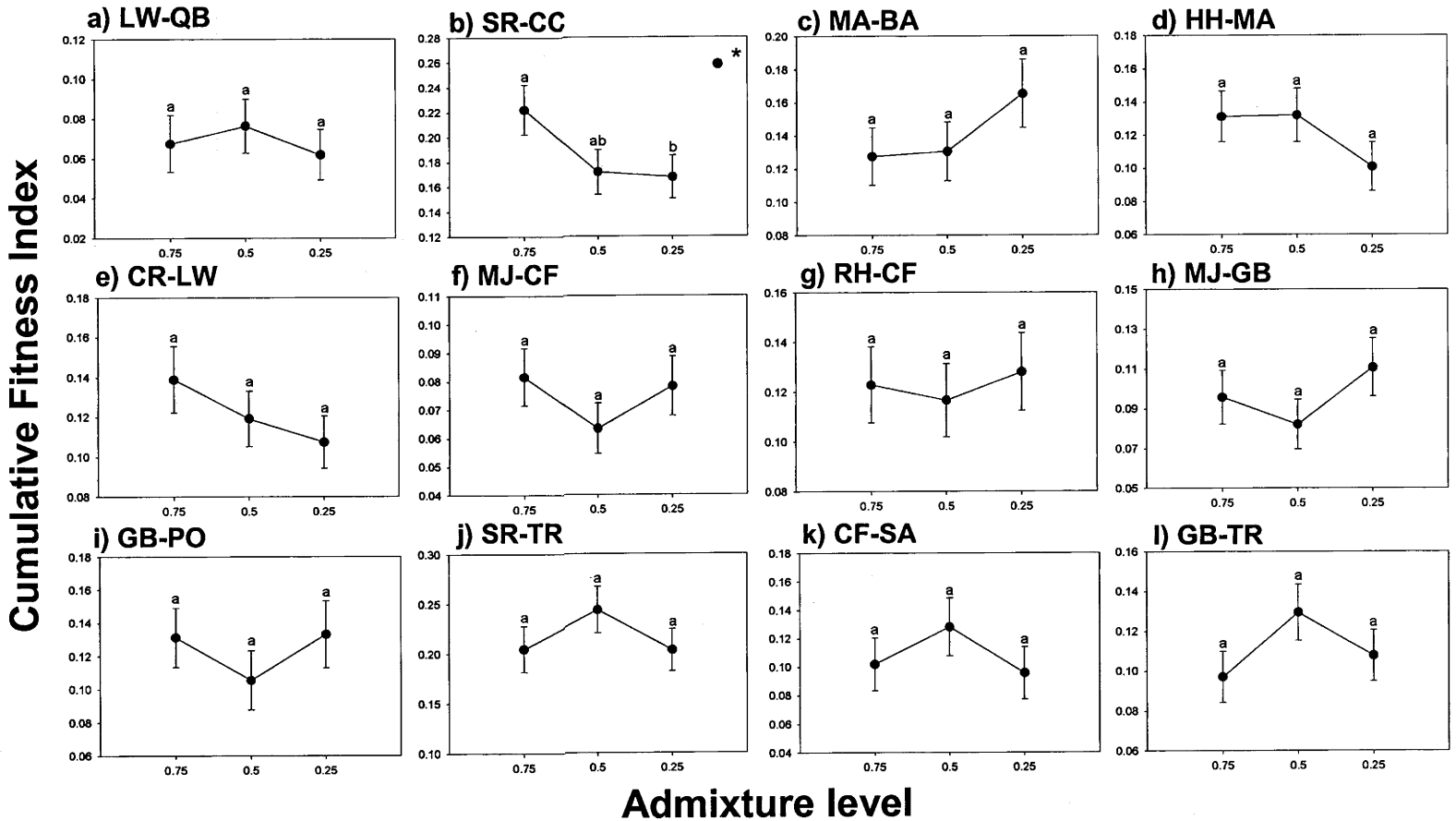


Figure 5.10 The difference in cumulative fitness index between the three levels of admixture ($BC_{F1xTARGET}$(75 % local genes), F_3 (50 % local genes) and $BC_{F1x\ SOURCE}$ (25% local genes)) in the second generation for12 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

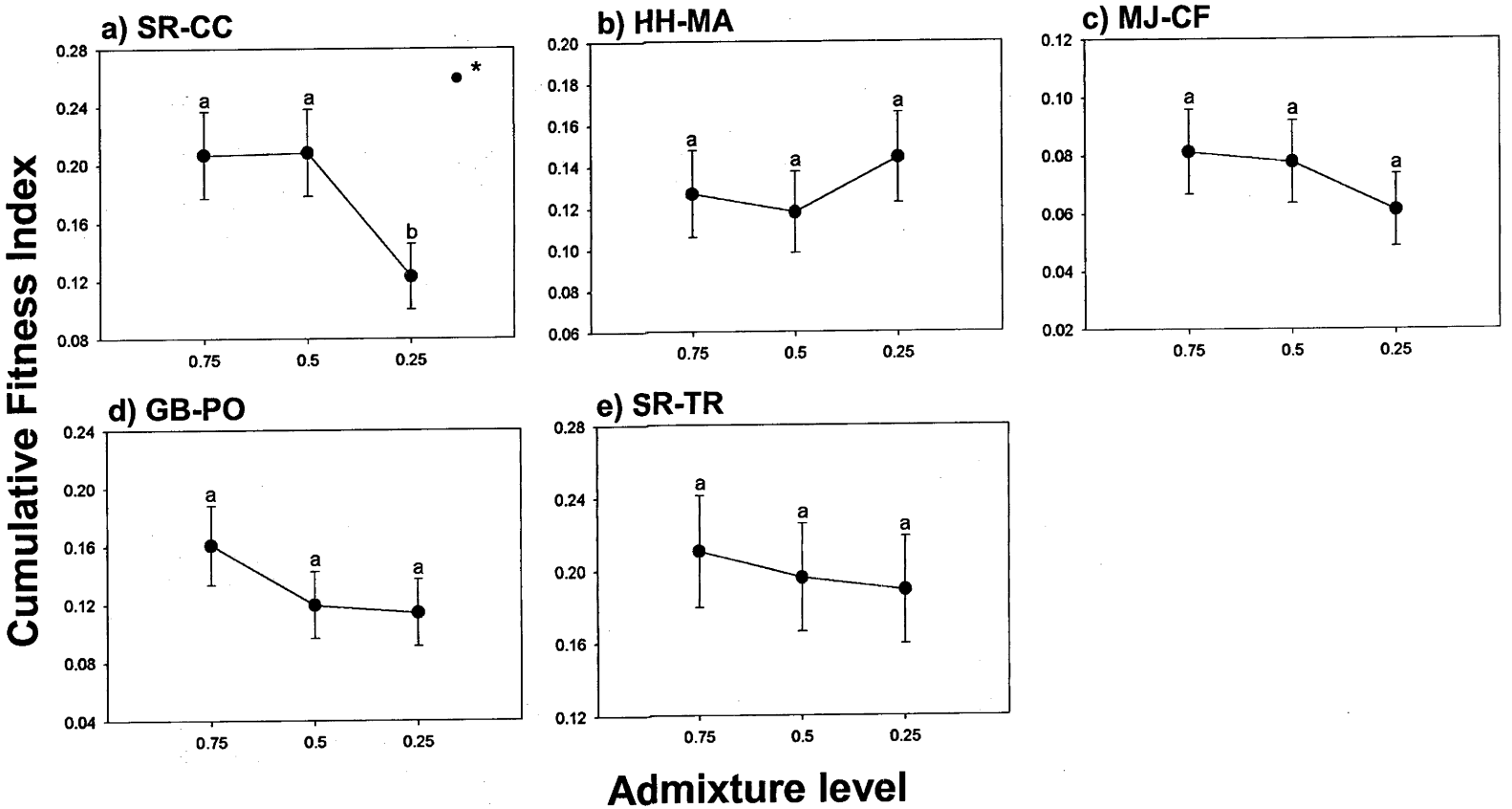


Figure 5.11 The difference in cumulative fitness index between the three levels of admixture ($BC_{F2xTarget}$ (75 % local genes), F_3 (50 % local genes) and $BC_{F2x\ Source}$ (25 % local genes)) in the third generation for 5 population pairs of *Rutidosis leptorrhynchoides*.

Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

Table 5.2 The results of the overall analysis at a set level of admixture (50 % local genes) for the F_1, F_2 (n = 12) and F_3 (n = 5) generations including all population pairs of *Rutidosis leptorrhynchoides* for a range of fitness traits across the lifecycle.

Wald statistics and *P* values represent the results of the global statistical model for GLM and REML analyses testing for significant differences between all crossing treatments. Comparisons between recombination levels represent subsequent LSD comparisons. For each fitness trait different letters indicate statistically significant differences between crossing treatments ($P < 0.05$). Values in parentheses represent one standard error.[1]Deviance ratio for GERM and CFI_{12}.

			Generation		
			Level of recombination		
Fitness trait	Wald/d.f.[1]	*P* value	F_1 (0)	F_2 (1)	F_3 (2)
Seedling					
Germination (GERM) (%)	9.38	<0.001	70.8 (1.3) [a]	76.0 (1.3) [b]	85.5 (2.5) [c]
Juvenile					
Number of leaves ($NoLVS_4$)	6.59	<0.001	11.9 (0.3) [a]	13.8 (0.3) [b]	14.1 (0.6) [b]
Rosette Height ($RosHT_4$) (mm)	7.05	<0.001	28.6 (0.4) [a]	30.8 (0.4) [b]	28.3 (0.9) [a]
Adult					
Number of leaves ($NoLVS_8$)	1.83	0.076	102.7 (1.6) [a]	107.4 (1.6) [a]	103.0 (3.6) [a]
Plant Height (HT_8) (mm)	3.94	<0.001	191.6 (2.4) [a]	190.2 (2.5) [a]	174.6 (5.3) [b]
Reproductive adult					
Total biomass (TB_{12}) (g)	1.71	0.101	2.65 (0.03) [a]	2.71 (0.03) [a]	2.61 (0.07) [a]
Number of flowers and buds ($NoF\&B_{12}$)	1.52	0.155	2.30 (0.08) [a]	2.37 (0.08) [a]	2.18 (0.16) [a]
Cumulative fitness index	3.82	<0.001	0.116 (0.01) [a]	0.129 (0.01) [a]	0.127 (0.01) [a]

5.3.2.2.2 Rosette height ($RosHT_4$)

Recombination level had a significant effect on $RosHT_4$ for the overall analysis including all population pairs ($P < 0.001$; Table 5.2) and within 2 of the 12 population pairs (Figure 5.13 b, g, l). For the overall analysis $RosHT_4$ increased significantly in the F_2, but then decreased in the F_3 so that there was no difference in $RosHT_4$ between the F_1 and F_3 generations (Table 5.2). For RH-CF there was a significant increase in $RosHT_4$ with increasing recombination between the F_1 and F_2 generations (Figure 5.13 g), while for SR-CC $RosHT_4$ significantly increased between the F_1 and F_2, but $RosHT_4$ in the F_3 was not significantly different from either the F_1 or F_2 generations.

5.3.2.3 Adult (8 months)

5.3.2.3.1 Number of leaves ($NoLVS_8$)

Recombination level had no significant effect on the mean $NoLVS_8$ for the overall analysis ($P > 0.05$; Table 5.2) and for 10 of the 12 individual population pairs (Figure 5.14 a, c – h, j – l). For the 2 significant population pairs (SR-CC (1.5 km) and GB-PO (78.9 km)) recombination level had a differential effect on $NoLVS_8$. For SR-CC (Figure 5.14 b) the mean $NoLVS_8$ significantly increased between the F_1 and F_3, while for GB-PO (Figure 5.14 i) the $NoLVS_8$ was significantly reduced in the F_3 compared to the F_1 and F_2 generations with lower levels of recombination.

5.3.2.3.2 Plant height (HT_8)

The level of recombination had a significant effect on HT_8 including all population pairs ($P < 0.001$; Table 5.2) and for 2 individual population pair comparisons (MJ-CF (27.8 km; Figure 5.14 f) and MJ-GB (71.9 km; Figure 5.14 h)). In the overall analysis mean HT_8 decreased significantly with increasing recombination between the F_2 and F_3 generations, but there was no difference in HT_8 between the F_1 and F_2 (Table 5.2). Both MJ-CF (Figure 5.14 f) and MJ-GB (Figure 5.14 h) showed declines in HT_8 as recombination increased, with a significant decrease in mean HT_8 between the F_1 and F_3 for MJ-CF and between the F_1 and F_2 generations for MJ-GB.

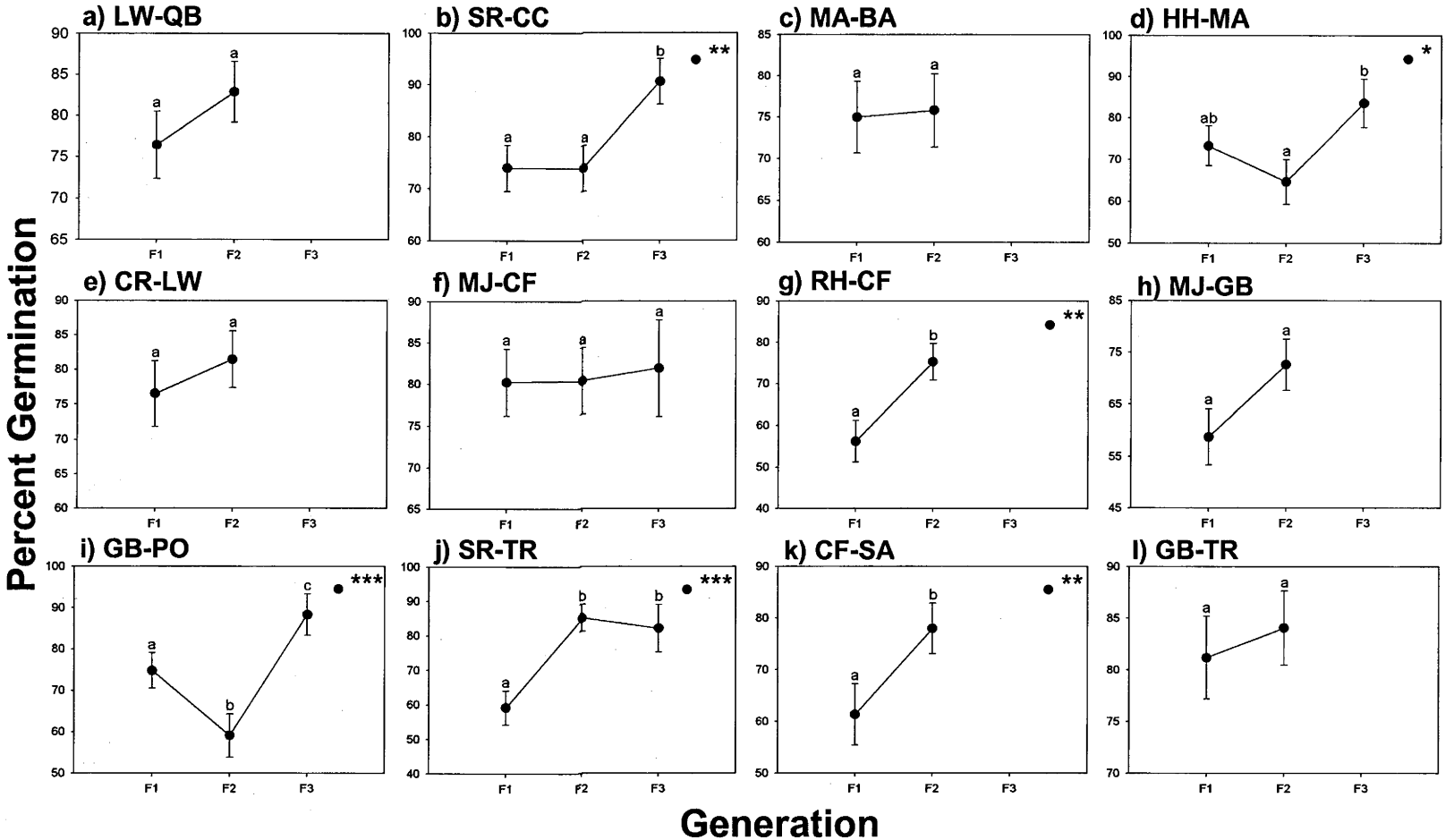


Figure 5.12 The difference in mean germination between the F_1, F_2 and F_3 generations for 12 population pairs of *Rutidosis leptorrhynchoides*.

Each generation has a set level of admixture of 50 % local genes, but varied in the level of recombination. Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error.
* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y axes vary between population pair comparisons.

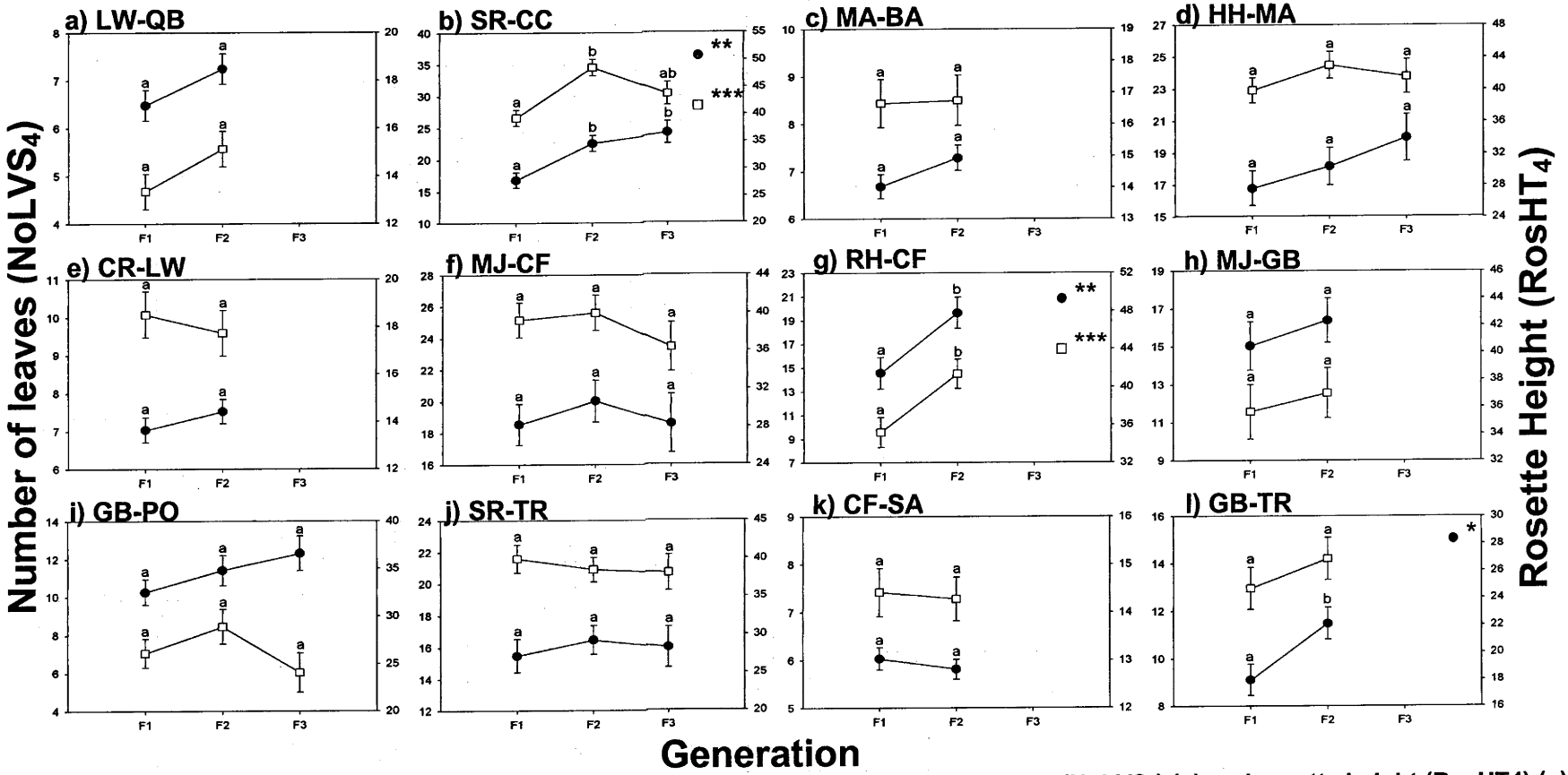


Figure 5.13 The difference between the F_1, F_2 and F_3 generations in the mean number of leaves ($NoLVS_4$) (●) and rosette height (RosHT4) (□) for 12 population pairs of *Rutidosis leptorrhynchoides*.

Each generation has a set level of admixture of 50 % local genes, but varied in the level of recombination. Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error.
* $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y axes vary between population pair comparisons.

5.3.2.4 Reproductive adult (12 months)

5.3.2.4.1 Total biomass (TB_{12})

There was no significant difference in TB_{12} between the different recombination levels for the overall analysis ($P > 0.05$; Table 5.2) and within 9 of the 12 individual population pairs (Figure 5.15 a – f, h, j, k). For 2 of the 3 significant population pairs (RH-CF (34.8 km; Figure 5.15 g) and GB-TR (586.2 km; Figure 5.15 l) mean TB_{12} increased significantly between the F_1 and F_2 generations. For GB-PO (78.9 km; Figure 5.15 i), however, there was no difference in TB_{12} between the F_1 and F_2, but there was a significant decrease in TB_{12} at the highest level of recombination in the F_3 generation.

5.3.2.4.2 Number of flowerheads and buds ($NoF\&B_{12}$)

Recombination level had no significant effect on the mean $NoF\&B_{12}$ for the overall analysis including all population pairs ($P > 0.05$; Table 5.2) or for any of the individual population pair comparisons spanning a range of geographic distances (Figure 5.15 a – l).

5.3.2.5 Cumulative fitness (CFI_{12})

There was no significant effect of recombination level on CFI for the overall analysis including all population pairs ($P > 0.05$; Table 5.2) or for 11 of the 12 individual population pairs (Figure 5.16 a – i, k, l). SR-TR was the only population pair where CFI differed between generations, with a significant increase in CFI between the F_1 and F_2, but no difference in CFI between these two generations and the F_3 (Figure 5.16 j).

5.3.3 Interaction between admixture and recombination

There was no interaction between admixture and recombination for the overall analysis including all population pairs for a range of fitness traits across the life-cycle (Appendix 5.1 and 5.2). However, there was a significant interaction between admixture and recombination in three population pairs for germination (SR-CC, GB-PO and SR-TR; Appendix 5.1) and a single population pair for HT_8 (SR-TR), TB_{12} (GB-PO), $NoF\&B_{12}$ (GB-PO) and CFI (HH-MA) (Appendix 5.2) suggesting that for some population pairs and fitness traits, the effect of admixture can change with the level of recombination and that these two mechanisms may act in concert to determine the fitness of hybrid progeny

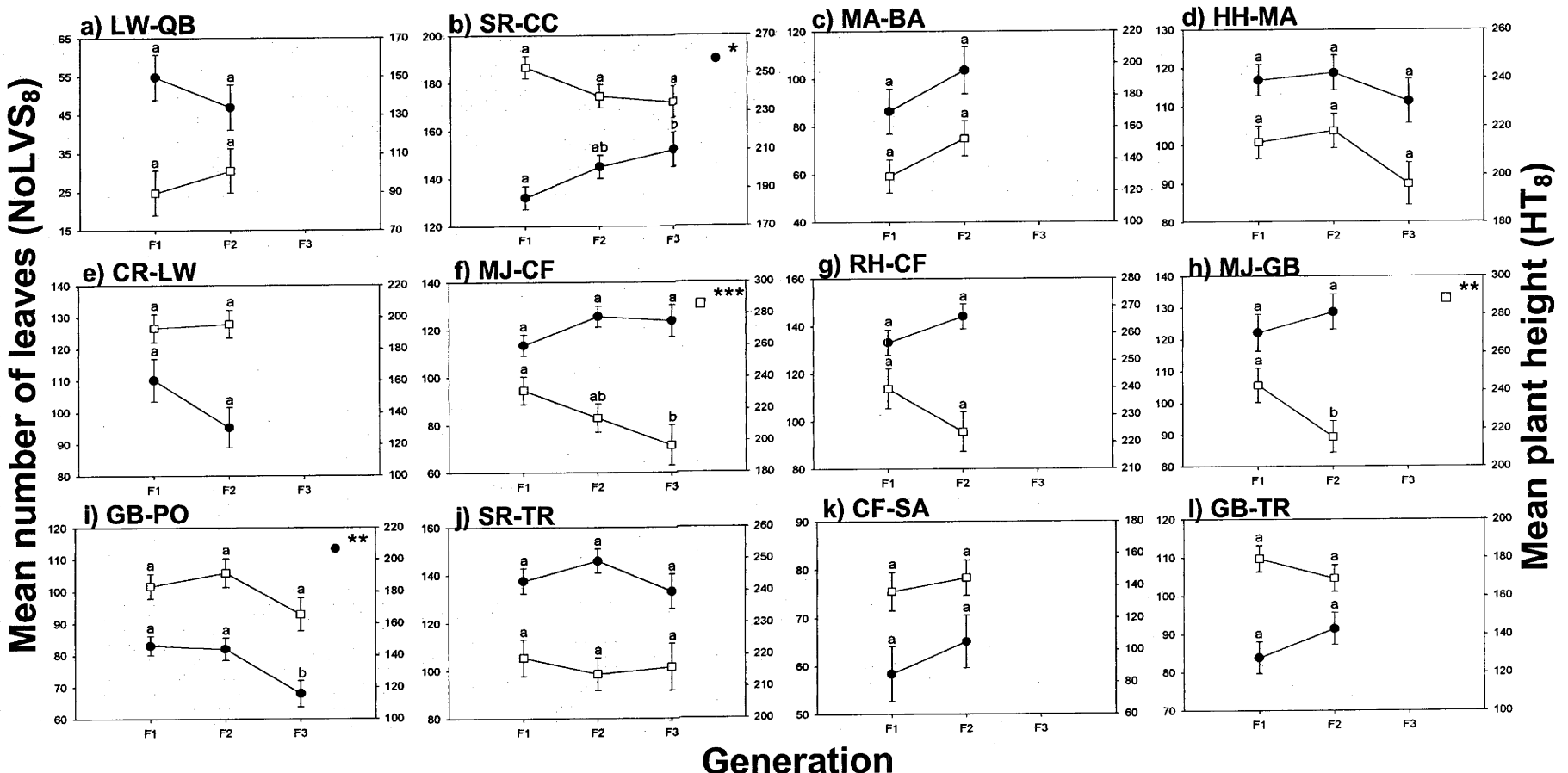


Figure 5.14 The difference between the F_1, F_2 and F_3 generations in the mean number of leaves ($NoLVS_8$) (•) and mean plant height (HT_8) (□) for 12 population pairs of *Rutidosis leptorrhynchoides*.

Each generation has a set level of admixture of 50 % local genes, but varied in the level of recombination. Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

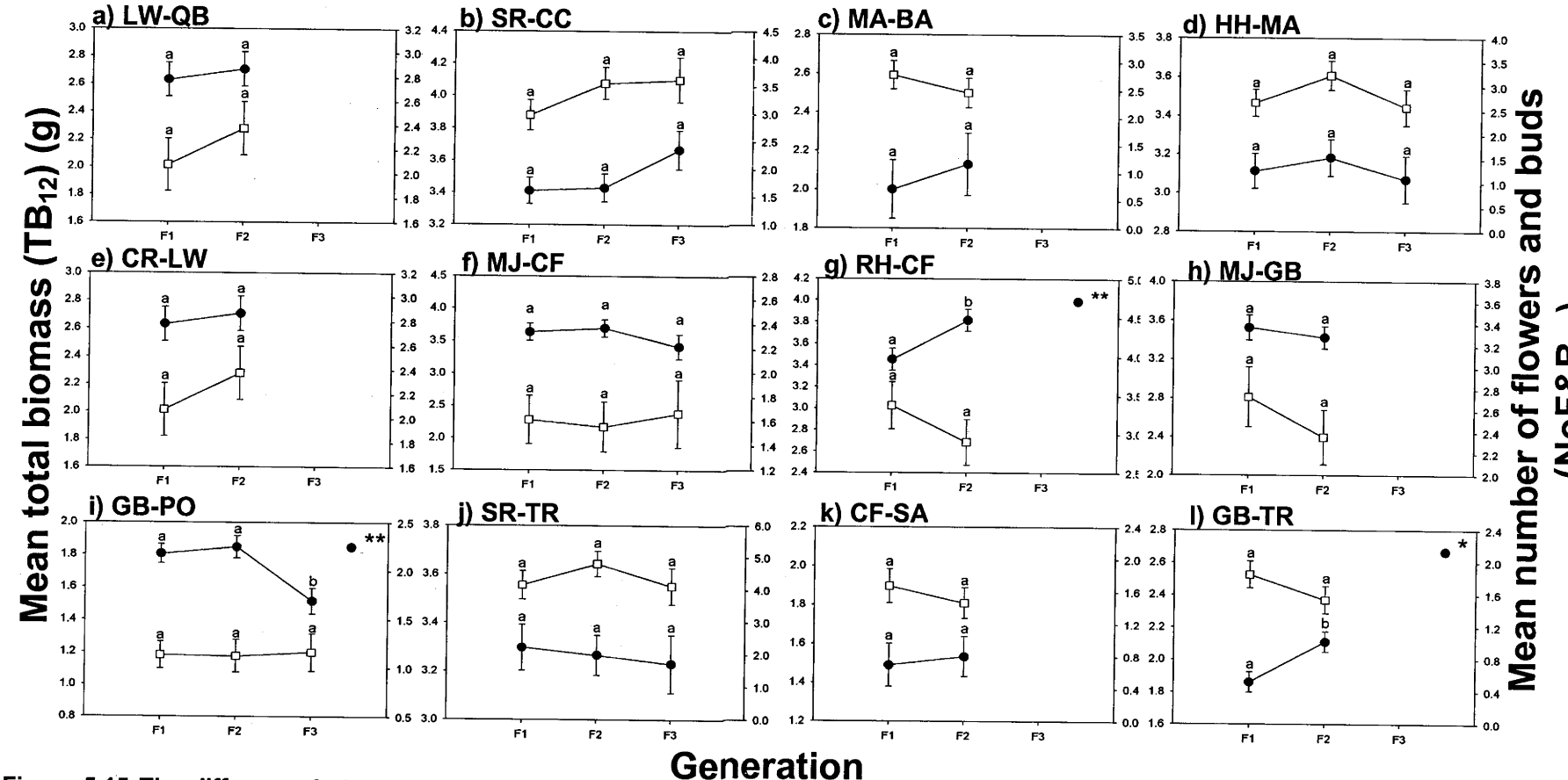


Figure 5.15 The difference between the F_1, F_2 and F_3 generations in mean total biomass (TB_{12}) (•) and the mean number of flowers and buds ($NoF\&B_{12}$) (□) for 12 population pairs of *Rutidosis leptorrhynchoides*.

Each generation has a set level of admixture of 50 % local genes, but varied in the level of recombination. Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

For example, for seedling germination there was a significant interaction between admixture and recombination for SR-CC ($P = 0.002$; Figure 5.17 a), GB-PO ($P = 0.032$; Figure 5.17 b) and SR-TR ($P = 0.041$; Figure 5.17 c) such that in all three population pairs the effect of admixture on germination was dependent on the level of recombination.

5.3.4 Cytoplasmic – nuclear epistasis

Cytoplasmic background (CB) had no effect on hybrid progeny fitness in the F_1 for a range of fitness traits across the lifecycle for the overall analysis including all population pairs (Appendix 5.3) and for 9 of the 12 individual population pair comparisons (Appendix 5.3). Two population pairs (SR-CC (1.5 km) and MJ-GB (71.9 km)) showed evidence of cytoplasmic-nuclear epistasis for a single fitness trait, with a significant increase in GERM for SR-CC (GERM local CB = 85.0 (± 1.8) %; foreign CB = 67.1 (± 1.5) %) and CFI_{12} for MJ-GB (CFI_{12} local CB = 0.10 (± 0.02); foreign CB = 0.05 (± 0.01)). MJ-CF (27.8 km) was the only population pair where there was a significant effect of cytoplasmic background in more than one fitness trait (Table 5.5) with greater mean plant height (HT_8 local CB = 259.6 (± 19.3) mm; foreign CB = 207.9 (± 16.3) mm) and reproduction ($NoF\&B_{12}$ local CB = 2.00 (± 0.25); foreign CB = 1.36 (± 0.22)) in F_1 progeny with a local cytoplasmic background. These results suggest that although there was some evidence for the positive contribution of cytoplasmic-nuclear epistasis to hybrid progeny fitness, for the majority of population pairs of *R. leptorrhynchoides* there was little differentiation for cytoplasmic-nuclear epistasis.

5.4 Discussion

Additive and non-additive modes of gene action are both important in determining the fitness of hybrid progeny for *R. leptorrhynchoides*, although the importance of each mechanism varied among population pairs and fitness traits, suggesting variation in the level of population differentiation and the differential expression of these two mechanisms across the life cycle. A comparison of different population pairs found that local adaptation had the greatest impact on germination, but in later life stages was often equivalent to the number of population pairs with positive effects of introducing foreign genes. Furthermore, recombination increased progeny fitness for germination and early

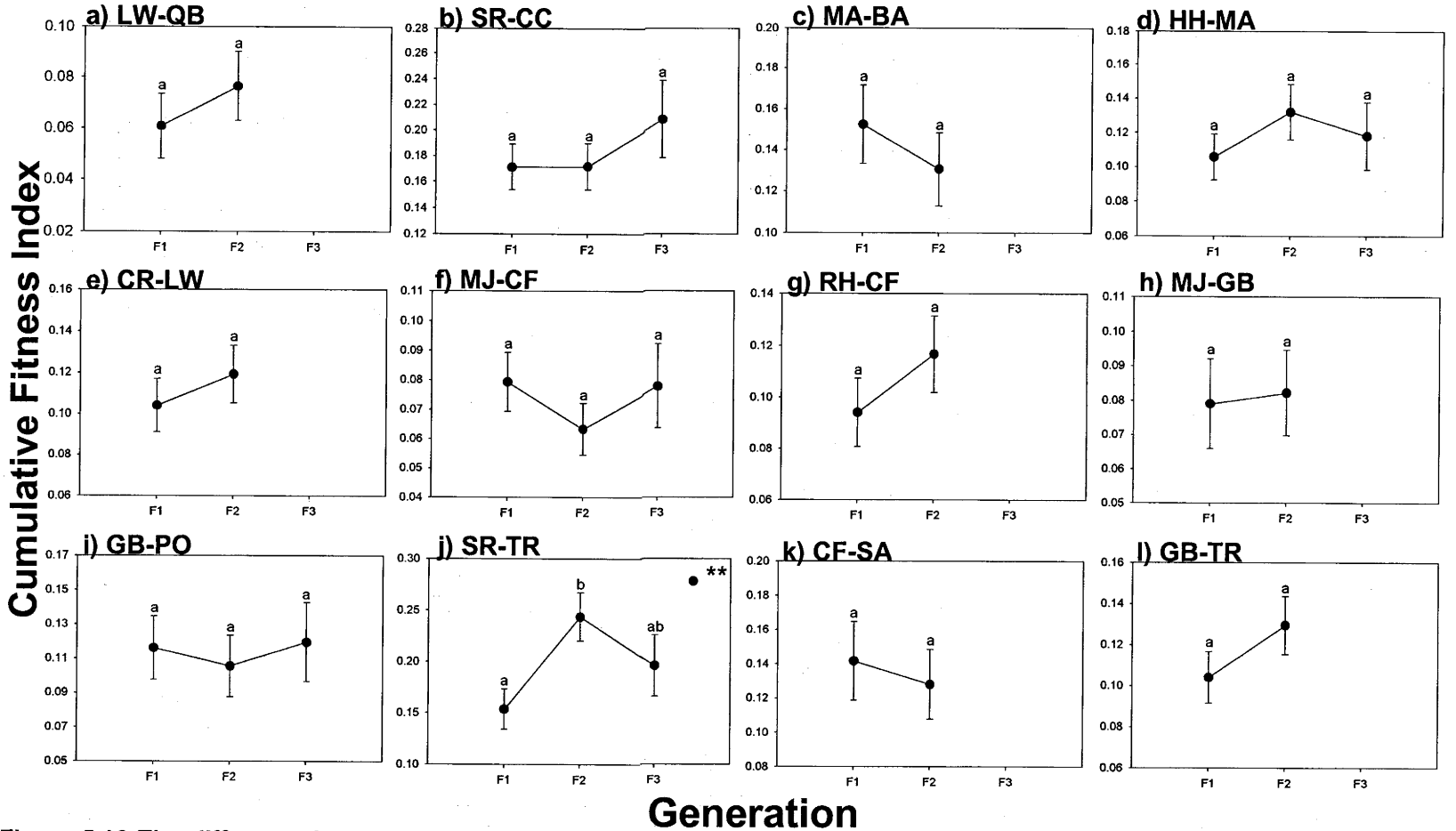


Figure 5.16 The difference in mean cumulative fitness index between the F_1, F_2 and F_3 generations for 12 population pairs of *Rutidosis leptorrhynchoides*.

Each generation has a set level of admixture of 50 % local genes. Population pairs are listed in order of increasing geographic distance between populations. Vertical bars represent ± 1 standard error. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Note: Scales on y-axis vary between population pair comparisons.

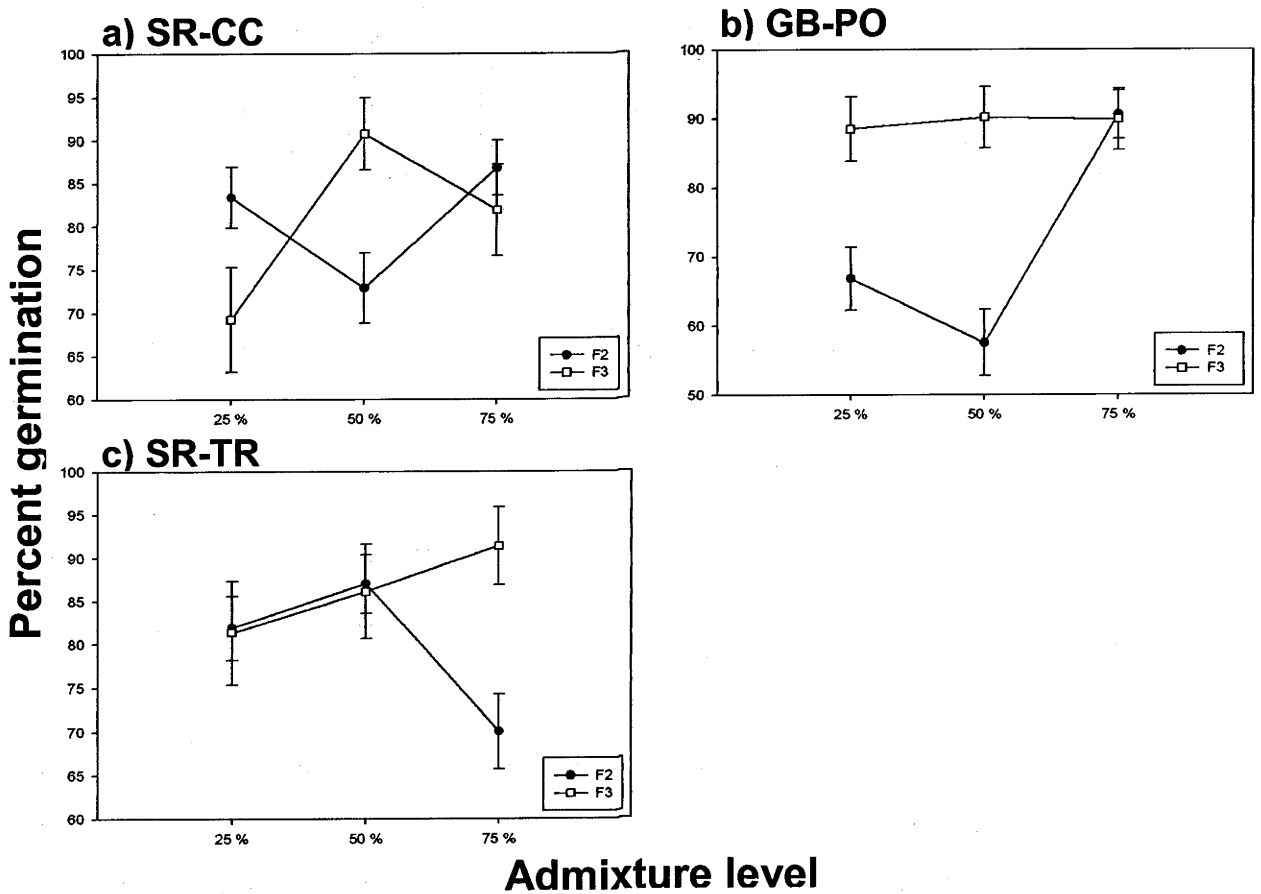


Figure 5.17 The effect of admixture level (25, 50 and 75 % local genes) and recombination (F_2 (•) and F_3 (□)) on percent germination for hybrid progeny from the three population pairs (a) SR-CC (1.5 km), (b) GB-PO (78.9 km) and (c) SR-TR (506.2 km) with a significant interaction term for these two factors (admixture *x* recombination).

Vertical bars represent ± 1 standard error.

seedling traits, but resulted in a decrease in fitness at the adult life stage for some traits. This implies that the strongest effect of both mechanisms was in early life history characters. Therefore, examining the contribution of both local adaptation and epistasis to the fitness of inter-population hybrids across the lifecycle can provide important insights into adaptive changes in genetic architecture and patterns of population differentiation in this species.

5.4.1 Local adaptation (admixture analysis)

The dilution of genes associated with local adaptation can reduce the fitness of inter-population hybrids, indicating adaptive population differentiation (Montalvo & Ellstrand 2001). In the overall analysis there was inter-generational variation in the effect of admixture on progeny fitness for *R. leptorrhynchoides*, with evidence of local adaptation for a number of traits (e.g. GERM and CFI_{12}) only in the third generation. The effect of admixture also varied between population pairs, with only one pair (SR-CC) showing consistent evidence of local adaptation across a range of fitness traits, while for other pairs local adaptation was only observed in one (e.g. RH-CF, SR-TR) or two (e.g. MJ-CF, GB-PO, CR-LW, HH-MA) fitness traits across the lifecycle. For cumulative fitness (CFI_{12}) local adaptation was only observed in SR-CC but was consistent in both the second and third generations. An interesting result of this study was the positive effect of increasing the proportion of foreign genes on a range of fitness traits across the lifecycle. In this case, an increase in plant growth and/or reproduction between 50 and 25 % admixture was observed for a number of population pairs (e.g. MA-BA, MJ-GB, CF-SA, GB-TR, GB-PO, SR-CC, MJ-CF) and may reflect the adaptive value of foreign genes or the indirect effects of release from bi-parental inbreeding in populations of this species.

Population divergence through adaptation to local environments has been observed in a number of plant species over a range of spatial scales (see Leimu & Fischer in review; Linhart & Grant 1996) suggesting a complex interaction between the evolutionary processes of selection, drift and gene flow (Kawecki & Ebert 2004; Slatkin 1987; Waser 1993) that is not necessarily related to geographic distance between populations. Like the present study, recent investigations of translocation and the fitness effects of inter-population hybridisation in field environments for *Chamaecrista fasciculata* have found

limited evidence for local adaptation (Galloway & Fenster 1999; Galloway & Fenster 2000), with adaptive differentiation only observed over large spatial scales (Galloway & Fenster 2000). In contrast, superior performance of local genotypes of *Papaver* and *Silene* (Keller et al. 2000) and a reduction in progeny fitness with increasing environmental differentiation for *Lotus scoparius* (Montalvo & Ellstrand 2001) provide support for the importance of local adaptation in these species. Limited evidence of adaptive differentiation in *R. leptorrhynchoides* across a number of populations, along with the presence of local adaptation at small spatial scales (i.e. for SR-CC (1.5 km)), suggests that although populations can differentiate in response to local selection regimes, genetic drift and/or gene flow may play a key role in counteracting the development of local adaptation in this species.

The importance of gene flow as an evolutionary process is well established (Arnold 1997; Lenormand 2002; Slatkin 1987) and the introduction of novel alleles through gene flow may play an important role in the adaptive evolution of populations (Lenormand 2002), as was demonstrated for *R. leptorrhynchoides* in the present study. In this case, the addition of new alleles in some population pairs produced adaptively relevant changes in plant fitness and resulted in enhanced fitness at higher levels of admixture. These results indicate that in the absence of local adaptation, the addition of novel alleles through gene flow may play an important role in adaptive evolution.

5.4.2 Co-adapted gene complexes (recombination analysis)

Recombination (due to either independent assortment of chromosomes or crossing over within chromosomes) changes the genetic background on which alleles are expressed (Brodie 2000). Therefore if favourable epistatic interactions develop in populations through drift and/or selection, then increasing recombination will result in an associated decline in fitness in recombinant hybrid progeny (Demuth & Wade 2005; Fenster et al. 1997). For inter-specific hybrids, the contribution of epistasis to hybrid fitness and genetic architecture is well established (e.g. Burke et al. 1998; Fishman & Willis 2001; Fritz et al. 2006; Gardner et al. 2000; Rhode & Cruzan 2005; Rieseberg et al. 1996) and can result in reproductive isolation and speciation due to the expression of Dobzhansky-Muller incompatibilities (Dobzhansky 1937; Muller 1940; Orr 1995; Orr & Turelli 2001) or conversely may contribute to adaptive evolution through the production of

highly fit transgressive segregants (Burke et al. 1998; Johansen-Morris & Latta 2006; Rieseberg et al. 1996). However, the lack of empirical studies examining the fitness effects of intra-specific hybridisation beyond the F_1 means that our understanding of the contribution of epistasis to adaptive divergence within species is limited.

The importance of epistasis to genetic architecture and population differentiation has been demonstrated in *C. fasciculata* where the negative fitness effects of recombination (through hybrid breakdown) were largely delayed until the F_3 (Fenster & Galloway 2000b). In addition, Keller et al. (2000) found reduced fitness in F_2 hybrid progeny for some fitness traits for all three species in this study, indicating hybrid breakdown and the loss of co-adaptation. Moreover, for the inter-tidal copepod *Tigriopus californicus*, there was a reduction in F_2 fitness through the loss of favourable gene interactions following recombination and this loss of fitness was associated with the level of population divergence (Burton 1990; Edmands 1999). However, the creative potential of recombination (Kaltz & Bell 2002) may increase hybrid progeny fitness, as was demonstrated for *C. fasciculata* where a small number of traits showed positive epistasis in the F_3 (Fenster & Galloway 2000b), while superior fitness was observed in the F_6 for lifetime fitness and biomass (Erickson & Fenster 2006) suggesting that recombination may facilitate adaptive evolution in this species. Furthermore, Bieri & Kawecki (2003) found epistasis to make a positive contribution to F_2 hybrid progeny fitness for the Cowpea Weevil (*Callosobruchus maculates*). In the current study, increasing recombination had both positive and negative effects on progeny fitness, depending on the particular fitness trait measured. For example in the overall analysis, increasing recombination between the F_1 and F_3 generations resulted in an increase in values of seedling and juvenile traits (GERM, $NoLVS_4$ and $RosHT_4$), while at the adult life-stage a significant decrease in HT_8 was observed in the F_3, suggesting the loss of co-adaptation. The differential effect of recombination on fitness traits across the lifecycle is also reflected in the individual population pair comparisons, with half the population pairs showing an increase in GERM between the F_1, F_2 and F_3 whereas only one or two populations showed an increase in fitness traits at the adult and reproductive adult life stage or for cumulative fitness (CFI_{12}). Furthermore, the greatest decrease in fitness with increased recombination was at the adult life stage, in which three population pairs

showed a reduction in fitness in later generations, suggesting the disruption of favourable epitasis.

The differential effect of recombination on fitness across the life-cycle for *R. leptorrhynchoides* suggests developmental changes in the importance of epistasis to genetic architecture and that overall co-adaptation may provide a minor contribution to population differentiation in this species. There are, however, a number of potential explanations for the enhancement in fitness with increasing recombination between the F_1, F_2 and F_3 generations, particularly in seedling and juvenile fitness traits. Firstly, as discussed above, recombination may produce favourable gene combinations (Erickson & Fenster 2006), although it is predicted that the stochastic production of highly fit hybrids through the recombination of novel genetic variation should increase the variance in fitness rather than the mean (e.g. Edmands 1999). In this study, however, there was no increase in the variance in fitness across generations, but instead mean fitness consistently increased. This suggests that while the creation of favourable gene combinations may contribute to the fitness of recombinant hybrid progeny, it is not the primary driver of enhanced fitness across generations. Secondly, restoration of parental genotypes through segregation in later generations may result in recovery from reduced fitness in the F_1 through underdominance or the loss of additive *x* additive epistasis. Yet as outlined in Figure 1.1, a recovery in fitness suggests that F_2 and F_3 fitness would approach that of the parental population (i.e. fitness would be restored to parental levels). But as shown in chapter 4 hybrid progeny fitness in the F_2 and F_3, especially for germination and juvenile traits, was consistently superior to the parental population, demonstrating that recombination increased fitness beyond that of the control and F_1. The final potential mechanism is the indirect genetic effects of maternal boosting (see section 1.3.3.6) which may result in increased hybrid fitness in the F_2 and F_3 generations. In this case, the favourable maternal genetic environment of the F_1 (due to heterosis) may result in enhanced fitness in F_2 progeny (e.g. Bieri & Kawecki 2003), this increase is then subsequently transmitted to the F_3 due to the superior genetic environment of the F_2, resulting in increased fitness across the generations. Maternal effects are predominantly expressed in early life stages (see Mousseau & Fox 1997; Roach & Wulff 1987) which is reflected in the current study where the greatest increase in progeny fitness between the F_1 and F_3 was in seedling and juvenile traits. Moreover,

the increase in fitness between the F_1 and F_3 is unlikely to be the result of selection across generations, since there was no difference in survival and/or germination between the randomly chosen seed used to generate F_1 maternal plants (data not shown) and each maternal line contributed equally to the next generation. Additionally, the maternal plant environment was constant for all generations in this experiment due to consistency in glasshouse, soil and nutrient conditions for all maternal plants in each generation.

5.4.3 Interaction of the genetic mechanisms underlying hybrid progeny fitness

The loss of local adaptation and disruption of co-adapted gene complexes are not mutually exclusive genetic mechanisms but may interact or act synergistically to influence the fitness of hybrid progeny (Lynch 1991). In this study, there was no significant interaction between admixture and recombination across all population pairs suggesting that these two mechanisms act independently to determine the fitness of inter-population hybrids. However, the importance of each genetic mechanism varied between fitness traits across the lifecycle, with a significant effect of both mechanisms on GERM and $RosHT_4$, while only one mechanism influenced hybrid progeny fitness for $NoLVS_4$, HT_8, TB_{12} and CFI_{12}. In contrast to the overall analysis, there was variation in the interaction of these two mechanisms between population pair comparisons, indicating that for some population pairs, especially in early life-history traits, non-additive modes of gene action may influence the expression of additive traits. The potential interaction between epistatic and additive effects (Brodie 2000; Lynch 1991; Lynch & Walsh 1998) has important implications for population divergence and, as suggested by the results of this study, may produce developmental changes in the fitness effects of inter-population hybridisation across the life-cycle.

5.4.4 Cytoplasmic – nuclear epistasis

Cytoplasmic-nuclear epistasis had been found to make an important contribution to population differentiation in a number of species (e.g. Demuth & Wade 2007; Edmands & Burton 1999; Galloway & Etterson 2005; Galloway & Fenster 1999; Galloway & Fenster 2001; Rawson & Burton 2002) and may have important implications for evolutionary divergence between populations (Demuth & Wade 2007; Galloway & Etterson 2005). For *R. leptorrhynchoides*, however, cytoplasmic background had very

little influence on F_1 hybrid progeny fitness across all population pairs and for the majority of the individual population pair comparisons, with evidence of cytoplasmic-nuclear epistasis in only three population pairs (SR-CC, MJ-CF and MJ-GB) for a limited number of fitness traits. The lack of cytoplasmic-nuclear epistasis is surprising given the potential for fine scale adaptive population differentiation for cyto-nuclear interactions due to high spatial structure (Wells & Young 2002) and low seed dispersal distances in this species (i.e. usually < 0.5 m; Morgan 1995a). However, this experiment was not designed to explicitly test cytoplasmic-nuclear epistasis. Therefore, low power to detect fitness differences between cytoplasmic backgrounds (with only six maternal lines per population pair) may mean that in some cases the lack of relationship may be due to low sample size, rather than the presence of adaptively relevant cytoplasmic-nuclear epistasis.

5.4.5 Genetic mechanisms and geographic and environmental distance

Local adaptation (Galloway & Fenster 2000; Linhart & Grant 1996; Montalvo & Ellstrand 2000; Waser & Price 1994) and differences in genetic architecture (Edmands 1999; Edmands 2002; Fenster & Galloway 2000b) are predicted to scale with environmental and geographic distance (as surrogates of adaptive and evolutionary divergence) due to the expected relationship between geographic distance and genetic isolation (isolation-by-distance) as well as environmental heterogeneity. For *R. leptorrhynchoides*, however, environmental differentiation and spatial scale had little power to explain variation between populations in the effect of both admixture and recombination.

In the present study there was no relationship between local adaptation and spatial scale, with a significant effect of admixture observed in population pairs spanning a range of geographic distances from 1.5 – 600 km. Furthermore, the only population pair with consistent evidence of local adaptation was SR-CC, which represents a very local spatial scale (1.5 km). The positive effect of increasing the proportion of foreign genes was also unrelated to geographic distance, with evidence of increased growth and/or reproduction at 50 and 25 % admixture in population pairs representing the full range of spatial scales. This lack of association between geographic distance and local adaptation is substantiated by two recent studies (Galloway & Fenster 2000; Montalvo & Ellstrand

2000) that found only a weak correlation between local adaptation and geographic distance over large spatial scales.

Environmental distance had very little power to explain the variability in local adaptation between populations, with evidence of adaptive differentiation in population pairs representing a range of environmental distances from 0.81 (GB-PO) – 3.92 (CR-LW) (full range = 0.56 – 4.04). In addition, SR-CC was in the bottom quartile of environmental distances (EnvDist = 1.09), yet was the only population pair with consistent evidence of local adaptation. However, there was some association between environmental distance and local adaptation at the adult life-stage, with 4 of the 6 population pairs with evidence of local adaptation from the upper two quartiles of environmental distance. This is substantiated by the results of Montalvo & Ellstrand (2001) who found a positive relationship between environmental distance and the disruption of local adaptation in inter-population hybrids. Population size has the potential to influence patterns of adaptive differentiation due to the increased importance of selection compared to drift in large populations (Barrett & Kohn 1991; Linhart & Grant 1996; see also section 1.3.5.4). However, there was no consistent effect of Target population size on admixture analysis, with local adaptation observed in both small (e.g. HH (HH-MA)) and large Target populations (e.g. SR (SR-CC)). These results suggest that the expression of local adaptation may vary between different fitness traits across the lifecycle and that the complexity in patterns of adaptive differentiation in *R. leptorrhynchoides* may be due to the interaction of a number of processes that act in concert to structure adaptive genetic variation.

The effect of recombination on plant fitness was also unrelated to spatial scale with both enhanced and reduced fitness across generations for population pairs separated by a range of geographic distances from 1.5 – 600 km. This indicates that the complexity of genetic architecture in this species is not associated with geographic distance, and that populations may be equally divergent across a range of spatial scales. Like the current study, a lack of correlation between spatial scale and genetic architecture has been demonstrated for a number species including *C. fasciculata* (Fenster & Galloway 2000b), the red flour beetle *Tribolium castaneum* (Demuth & Wade 2007) and the Pitcher-Plant Mosquito *Wyeomyia smithii* (Lair et al. 1997). Although Edmands (1999)

found geographic and genetic distance to be a good predictor of F_2 hybrid progeny fitness for the inter-tidal copepod *T. californicus*.

Greater heterosis is expected following hybridisation between intermediately divergent populations (e.g. Moll et al. 1965). Yet for *R. leptorrhynchoides* the effects of maternal boosting in early seedling characteristics were found in population pairs spanning a range of distances (see Figure 5.12 and 5.13) suggesting very little correlation between population divergence and geographic proximity. Furthermore, as discussed above, an increase in the importance of drift in small populations (Barrett & Kohn 1991) may result in greater differentiation for genetic architecture in small compared to large populations. Consequently, small populations may show greater declines in fitness with increasing recombination. However, for *R. leptorrhynchoides*, evidence of the loss of co-adaptation with increasing recombination in adult traits was only found in large Target populations (e.g. MJ (MJ-CF and MJ-GB) and GB (GB-PO)) which suggests that drift plays a relatively minor role in structuring differences in genetic architecture in this species.

5.4.6 Conclusions

The results of this study indicate four important conclusions regarding the potential mechanisms underlying the fitness effects of admixture and recombination in *R. leptorrhynchoides*:

1. Heterosis in the F_1 can result in an increase in fitness that is subsequently passed on to the F_2 and F_3 via maternal boosting, especially for seedling and early life history traits.
2. This increase in fitness through maternal boosting may be counteracted by the breakdown in coadaptation in some population pairs for later life-history traits.
3. The chance creation of favourable gene combinations through recombination may contribute to increased fitness in later generations.
4. Although there was evidence of local adaptation in some pairs (e.g. SR-CC), increased fitness at higher levels of admixture (i.e. greater proportion of foreign genes) indicates the potential adaptive value of foreign genotypes.

Taken together, these results indicate that patterns of adaptive differentiation and genetic architecture in *R. leptorrhynchoides* are determined by a complex range of interacting processes, and that population size along with surrogates of adaptive and evolutionary divergence have little power to predict patterns of population differentiation in this species.

CHAPTER 6:

POPULATION SIZE, SELF-INCOMPATIBLITY AND GENETIC RESCUE IN DIPLOID AND TETRAPLOID POPULATIONS OF *Rutidosis leptorrhynchoides* (ASTERACEAE)

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6.1 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 & Tabah 2003; Mable et al. 2003). Self-incompatibility 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 & 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 break-down 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 e.g. *Aster furcatus* (Reinartz & Les 1994); *Scalesia affinis* (Nielsen et al. 2003). Fluctuations in SI in response to dramatic declines in population

size associated with colonisation dynamics has 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 (e.g. Good-Avila & Stephenson 2002; Hiscock 2000) or by the action of a cryptic gametopytic SI system (Hiscock 2000; Lewis et al. 1988). However, the strength of inbreeding depression is an important factor that determines the maintenance of SI (Brennan et al. 2005; Levin 1996), 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 colonisation 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 & 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 bi-parental 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 inter-plant variance in paternal fitness, as individuals with unique *S* genotypes are selected for (Byers & 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 co-dominance 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 (Castric & Vekemans 2004; Vekemans et al. 1998), 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 micro-sites or negative density dependence (e.g. 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 autoploypoloids) are expected to maintain higher genetic diversity (Bever & Felber 1992), and this has been demonstrated empirically for neutral loci for intra-specific comparisons (Brown & Young 2000; Hardy & Vekemans 2001; Mahy et al. 2000) and between autotetraploid and closely related diploid species (Hokanson & 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 fertilisation 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 polyploid species may be

expected. These two processes may also interact to alter mate limitation and the response of polyploidy 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 (e.g. Brennan et al. 2006; Karron et al. 1990; Kowyama et al. 1994; Young et al. 2000b), 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 (Glemin et al. 2005; Mable et al. 2003; Schierup et al. 2006) and Solanaceae (Richman et al. 1996)). Alternatively, a probability-based approach to estimate the likelihood of fertilisation 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 fertilisation. 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 fertilisation within a population relate to population size?
2. Does fertilisation 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?

6.2 Materials and methods

6.2.1 *Rutidosis leptorrhynchoides*

Rutidosis leptorrhynchoides is an insect pollinated multi-stemmed herbaceous perennial with a sporophytic self-incompatibility 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 ha since the mid 1800's (Brown & Young 2000; Kirkpatrick et al. 1995). 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° 30′S, > 148° 30′E) in New South Wales and the Australian Capital Territory, and a southern zone that extends through central Victoria (> 37°S, > 145° 30′E) (Brown & 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 & Young 2000; Murray & Young 2001).

Rutidosis leptorrhynchoides 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 & 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 & 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 know about the levels of inbreeding in populations, although dominance among *S*-alleles in sporophytic SI systems and high spatial genetic structure (Wells & Young 2002) may result in bi-parental inbreeding in this species.

6.2.2 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 to 95 000 reproductive plants) and geographic distances (0.7 to 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 x 10 m or 30 x 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. leptorrhynchoides* 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.

6.2.3 Pollination experiments and fertilisation success

To examine fertilisation 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 (i.e., 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 1/3 potting mix, 1/3 sand and 1/3 peat moss and grown in glasshouse conditions with temperatures maintained between 15 – 28°C. To ensure adequate flowering during the winter months, natural light was supplemented with artificial light to ensure a 14-hour 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 then 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 6. Although cross-pollinations with up to 6 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-fertilisation. Hence for each reciprocal cross there were 3 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 used to calculate the probability of fertilisation $P(F)$ both within (WI-POP) and between (BW-POP) populations;

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

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 fertilisation (DIFF FERT = BW-POP FERT– WI-POP FERT) which represents the increase in fertilisation success by crossing between populations.

6.2.4 Data analysis

The relationship between population size and; (a) the within population probability of fertilisation (WI-POP FERT), and (b) the difference in the probability of fertilisation (DIFF FERT) for diploid and tetraploid population pairs was analysed using multiple linear regression in Genstat (9th Ed.) 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 non-significant 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 (ANCOVA) 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.

6.3 Results

There was a significant positive relationship between reproductive population size (log) and within-population probability of fertilisation 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 6.1). For both chromosome races, there were substantial reductions in the probability of successful fertilisation 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 fertilisation rates as low as 50 - 60 %. The slope of the regression line was slightly greater for the tetraploids compared to the diploid populations (ß = 0.154 and ß = 0. 109 respectively), but there was no statistically significant difference between these two slopes ($P > 0.1$), indicating that the probability of fertilisation in diploid and tetraploid populations declines at a similar rate as population size deceases.

There was also no significant difference in the intercept of the two regression lines for diploid and tetraploid populations ($P > 0.1$)

Inter-population crossing generally resulted in increases in fertilisation 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 6.2). The difference in slopes between the two regression lines was substantial, with the slope for the tetraploid population pairs (ß = -0.144) more than double that for the diploid population pairs (ß = -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 fertilisation success of crossing between populations decline at a faster rate with increasing population size compared to diploid populations.

6.4 Discussion

Genetic diversity at the *S*-locus has important demographic consequences for small populations (Byers & Meagher 1992; Young et al. 2000b). A decline in fertilisation 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 fertilisation 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 fertilisation success was in small populations with low *S*-allele diversity. For tetraploid populations, however, the benefits to fertilisation success of crossing between populations declined

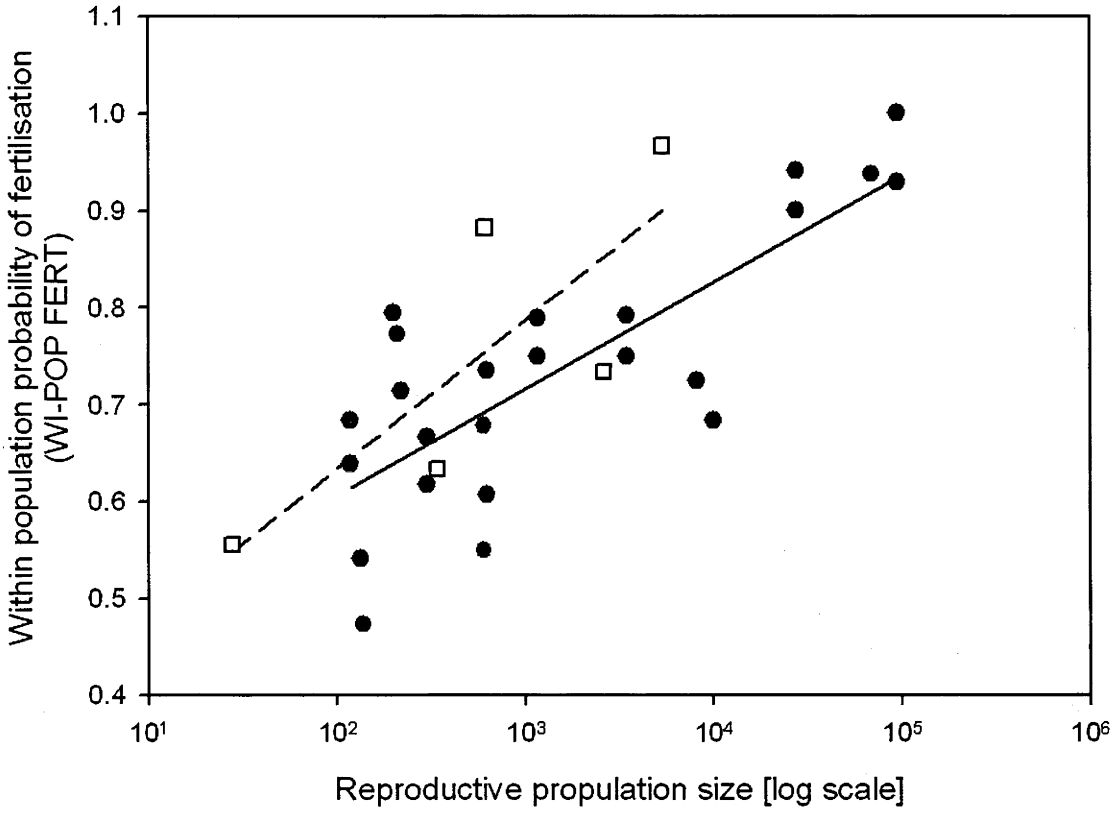

Figure 6.1 Within population probability of fertilisation as a function of reproductive population size (log scale) for diploid (●) (R^2 = 0.62; P < 0.001) and tetraploid (□) (R^2 = 0.52; P = 0.10) populations of *Rutidosis leptorrhynchoides*.

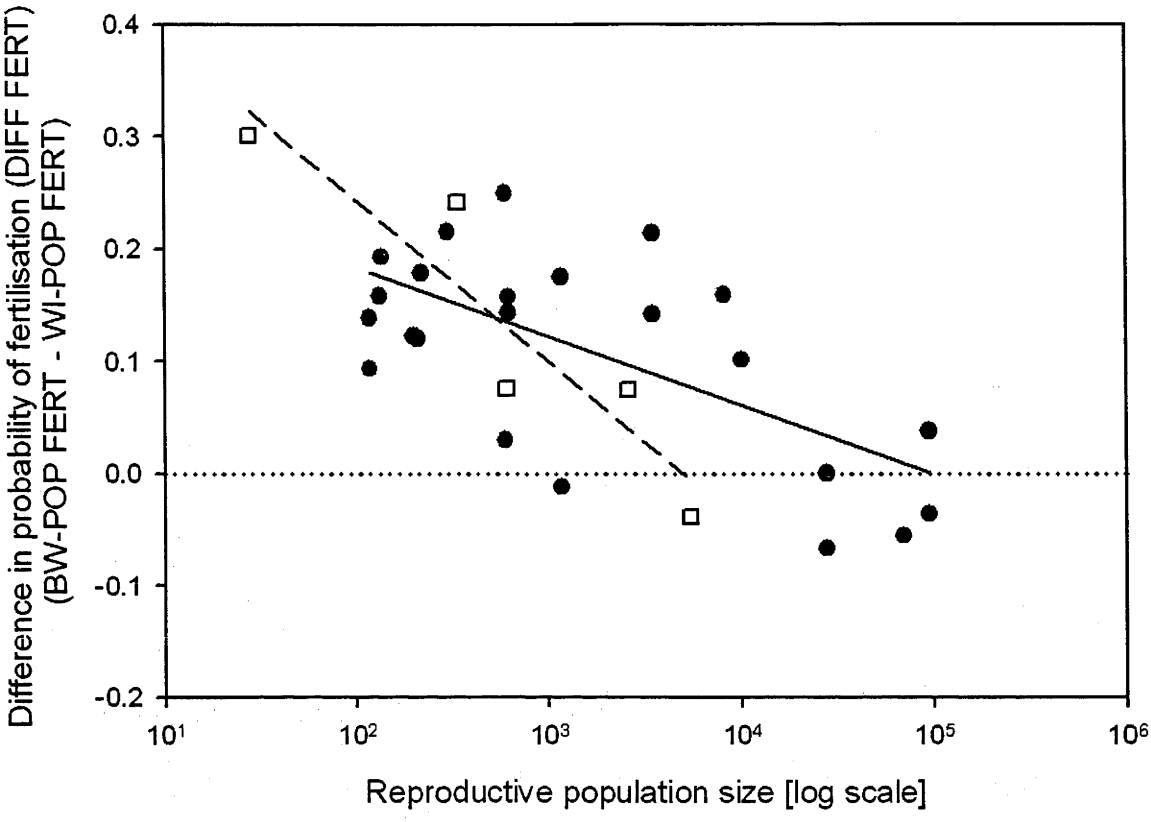

Figure 6.2 Difference in the probability of fertilisation (between population probability of fertilisation – within population probability of fertilisation) as a function of reproductive population size (log scale) for diploid (●) (R^2 = 0.41; *P* <0.001) and tetraploid (□) (R^2 = 0.80; *P* < 0.001) populations of *Rutidosis leptorrhynchoides*.

The dashed horizontal line represents where the difference in the probability of fertilisation was zero.

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 with 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 fertilisation success (Byers & 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 (Castric & Vekemans 2004; Vekemans et al. 1998). 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. 2000b; Young et al. 1999). The effect of drift on *S*-allele diversity, however, will depend on dominance relationships in the SI system (Castric & Vekemans 2004; Schierup et al. 1997). 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 fertilisation success and mate availability in these systems.

Polyploidy is predicted to have a buffering effect on allelic richness (Bever & Felber 1992; Brown & Young 2000), which has been demonstrated empirically for neutral loci in a number of species (Brown & Young 2000; Hardy & Vekemans 2001; Mahy et al.

2000; Soltis & Reiseberg 1986; Young et al. 2000b). 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 fertilisation 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 fertilisation 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 inter-population crossing. The first is that, on average, tetraploid populations could be more genetically differentiated for *S*-alleles than diploid populations. This would mean that inter-population 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 (Castric & Vekemans 2004; Schierup et al. 2000).

A second possibility is that small tetraploid populations are actually more mate limited than their diploid counterparts due to either: i) increased probability of matching alleles between tetraploid genotypes or, ii) 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 fertilisation 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 fertilisation success between diploids and tetraploids (Figure 6.1), 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.

Inter-population 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 & Fischer 2005). The predicted demographic outcomes for small populations with reduced *S*-allele diversity include lower and more variable seed set (Byers & Meagher 1992), which are trends that have been observed in small populations of a number of self-incompatible species (Fischer et al. 2003; Luijten et al. 2000; Widen 1993), including *R. leptorrhynchoides* (Morgan 1999). Recent studies to examine the relationship between population size and fertilisation 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, inter-population crosses significantly increased cross-compatibility which was reflected in higher seed set for between-population compared to within-population crosses (Willi & Fischer 2005). These results support the findings of this study that small populations experience the greatest increase in fertilisation 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 & Fenster 2000). Moreover, inter-population crosses may also result in heterosis, particularly for small populations suffering from inbreeding (e.g. Dudash & Fenster 2000; Willi & Fischer 2005). Therefore, rather than a trade-off, introducing new genetic material into small populations may result in a two-fold benefit to population viability; firstly from the benefits to fecundity through the restoration of *S*-allele diversity, and secondly any fitness gains accrued from heterosis.

CHAPTER 7:
GENERAL DISCUSSION

7.1 Summary of research findings

In a conservation context, local adaptation and outbreeding depression are central to decisions regarding the delineation of appropriate seed sourcing zones (Hufford & Mazer 2003; Montalvo & Ellstrand 2001) and the value of augmenting small or declining populations to increase population size, restore genetic diversity or counteract the deleterious effects of inbreeding depression (Fenster & Galloway 2000a; Moritz 1999). Outbreeding depression and an assessment of the underlying genetic mechanisms are also fundamental to understanding the evolutionary processes of adaptation, population divergence and speciation (Fenster & Galloway 2000b), and can provide important insights into the role of selection, gene flow and drift in population differentiation and genetic architecture.

The aim of the research presented in this thesis was to examine the consequences of translocation and inter-population hybridisation for *R. leptorrhynchoides* through an evaluation of; (1) local adaptation and patterns of adaptive differentiation (Chapter 3), (2) outbreeding depression over a number of generations (Chapter 4), (3) the relative importance of the two primary genetic mechanisms underlying outbreeding depression (Chapter 5), (4) the potential benefits to fertilisation success of introducing new genetic material (Chapter 6), and (5) the power of spatial scale, environmental distance (Chapter 2) and Target and Source population size to predict the genetic and demographic outcomes of translocation and inter-population hybridisation as mediated by these interacting processes.

This final chapter summarises the key findings and implications of this research for our understanding of the importance of outbreeding depression in fragmented plant populations, the evolutionary processes underlying population divergence and the management of remnant populations of *R. leptorrhynchoides*.

7.1.1 The importance of local adaptation and outbreeding depression for *Rutidosis leptorrhynchoides*

The primary conclusion of the local adaptation experiment is that, across a range of vegetative and reproductive fitness traits, there was no consistent evidence of local adaptation in *R. leptorrhynchoides*. For the majority of population pairs, local and foreign genotypes showed equivalent perfromance, while a minority of pairs showed either local adaptation or foreign genotype advantage across a range of spatial scales from 1 – 600 km (chapter 3). This variability in local adaptation was also found in the overall analysis including all population pairs, with equal performance of local and foreign genotypes, local adaptation and foreign genotype advantage all observed across a range of traits. For traits of greatest demographic importance (i.e. high elasticity traits), local adaptation was found for seedling survivorship and above ground biomass (as a surrogate of adult survivorship), while flowering stems showed an overall foreign genotype advantage. This suggests that translocation may have both positive and negative outcomes for mean population fitness, and that differences in performance between local and foreign genotypes may vary at different life stages. However, when fitness was assessed across the life cycle from germination through to reproduction (cumulative fitness), there was evidence of an overall foreign genotype advantage, indicating superior performance of the foreign Source populations (chapter 3). These results imply that even when translocating over large spatial scales (up to 600 km), foreign genotypes may have equivalent or greater performance than the local Target population.

The most important result of the outbreeding depression experiment was that in the majority of individual population pairs and for the overall analysis, the fitness of inter-population hybrids was either equal to or greater than the local parental (Target) population for a range of fitness traits across the life-cycle. There was, however, variability in the fitness effects of inter-population hybridisation among different population pairs as well as across generations and between fitness traits, with greatest heterosis in the F_2 and backcross generations for germination, juvenile growth and cumulative fitness.

For high elasticity traits, including seedling survivorship, total biomass (as a surrogate for adult survival) and reproduction, as well as for cumulative fitness across the lifecycle, inter-population hybridisation had no negative effects on progeny fitness in the overall analysis including all population pairs. Instead, heterosis was observed for hybrid progeny fitness (particularly in the F_2 and backcross generations) for the overall analysis of seedling survivorship and cumulative fitness and in a number of individual population pairs. These results indicate that inter-population hybridisation may potentially benefit long-term population viability and that fitness benefits may accumulate through the lifecycle, even when outcrossing over large spatial scales of up to 600 km. Moreover, for the two population pairs where significant outbreeding depression was observed for a number of traits (i.e. SR-CC and HH-MA), cumulative fitness was either equal to or greater than the parental populations, suggesting that outbreeding depression was not consistent over the lifecycle and that heterosis in some traits counteracts outbreeding depression in these population pairs.

7.1.2 Genetic mechanisms underlying outbreeding depression and the fitness effects of inter-population hybridisation

The overall fitness outcomes of inter-population hybridisation are determined by the complex interplay of a number of genetic mechanisms representing both additive and non-additive modes of gene action. The results of this study indicate six important conclusions regarding the potential mechanisms underlying the fitness effects of admixture (diluting genes associated with local adaptation) and recombination (disruption of favourable epistasis) in *R. leptorrhynchoides*: (1) Heterosis in the F_1 can result in an increase in fitness that is subsequently passed on to the F_2 and F_3 via maternal boosting, especially for seedling and early life history traits. (2) This increase in fitness through maternal boosting may be counteracted by the breakdown in coadaptation in some population pairs for later life-history traits. (3) The stochastic creation of favourable gene combinations through recombination may contribute to increased fitness in later generations. (4) Local adaptation contributes to fitness in some population pairs, but only for some traits. (5) Yet greater fitness at higher levels of admixture (i.e. greater proportion of foreign genes) indicates the potential adaptive value of foreign genotypes, and (6) Population size along with surrogates of adaptive

and evolutionary divergence (geographic and environmental distance) had little power to predict patterns of population differentiation in this species.

Figure 7.1 and the following section outlines the results of this thesis in the context of the potential genetic mechanisms underlying the fitness of the parental populations involved in translocation, and each generation following inter-population hybridisation in *R. leptorrhynchoides* as evidenced by these experimental results.

Parental populations

Local adaptation[1]: There was evidence of local adaptation in some population pairs and in the overall analysis for seedling survivorship and above ground biomass. However, there was also evidence of foreign genotype advantage through the superior performance of foreign genotypes in a number of population pairs and in the overall analysis for plant height, flowering stems and cumulative fitness (chapter 3).

Bi-parental inbreeding[2]: Greater heterosis in small Target populations following inter-population hybridisation provides some evidence for bi-parental inbreeding in small parental populations (chapter 4).

F_1, F_2 and F_3

Heterosis[3]: It is predicted that heterosis would be greatest in the F_1 due to inter-population hybridisation counteracting the deleterious effects of inbreeding in parental populations. In this study, however, the increase in fitness following inter-population hybridisation was greatest in the F_2 (chapter 4). This suggests that other factors such as the loss of **additive *x* additive epistasis**[4] or **underdominance**[5] may initially counteract the positive fitness effects of inter-population hybridisation, leading to the equivalent performance of F_1 progeny compared to the local parental population (chapter 4). Furthermore, the greatest increase in fitness in the F_2 indicates that **heterosis**[3] carries through to the subsequent generations and that other factors such as **maternal boosting**[6] (chapter 5) and/or the production of **advantageous recombinants**[7] (chapter 5) may also contribute to the higher fitness of F_2 progeny compared to the local parental population and the F_1. The reduction of fitness in the F_3 compared to the F_2 (chapter 4) also suggests that, although **heterosis**[3] may carry through from the F_1, the positive effects of heterosis decline in subsequent generations.

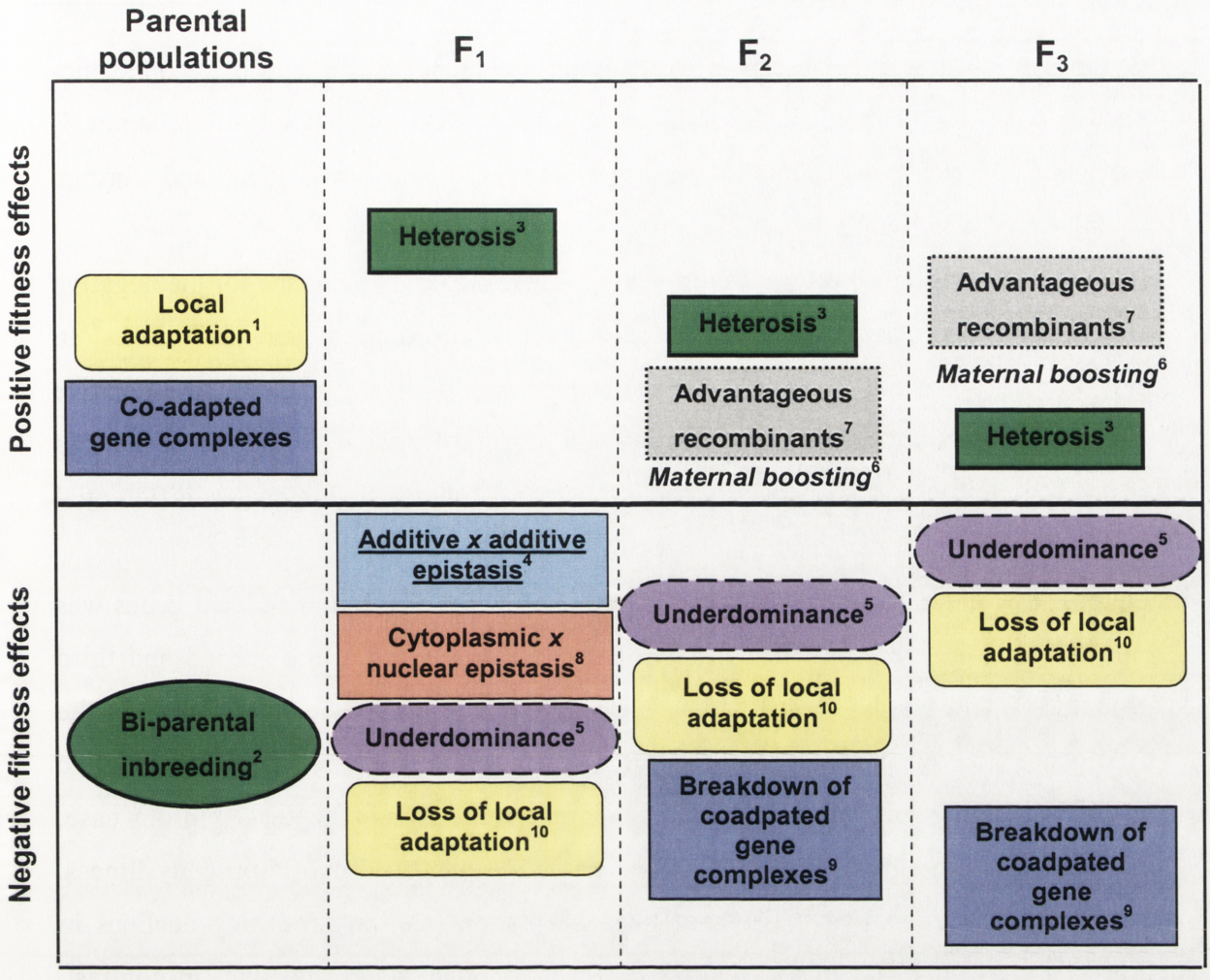

Figure 7.1 Potential genetic mechanisms that can influence fitness in the parental populations and the F_1, F_2 and F_3 following inter-population hybridisation.

Mechanisms above the solid line indicate positive fitness consequences, while those below the line indicate negative fitness consequences. The position above or below the line is not representative of the actual magnitude of the effect, however a change in the position of a mechanism in the F_1, F_2 or F_3 is indicative of the relative change in the potential importance of that mechanisms across generations.

Cytoplasmic-nuclear epistasis[8]: There was little evidence of the loss of cytoplasmic-nuclear epistasis for most fitness traits in the majority of population pairs (chapter 5) based on the lack of fitness differences between plants with local and foreign cytoplasmic backgrounds.

Breakdown of co-adapted gene complexes[9]: There was only evidence for the negative effects of recombination through the breakdown of **co-adapted gene complexes**[9] in later life history traits (e.g. plant height) (chapter 5). This suggests that the positive effects of **maternal boosting**[6] on seedling and juvenile fitness may counteract the loss of coadaptation in early life history traits (chapter 5), which highlights the differential importance of each mechanism across the lifecycle.

Loss of local adaptation[10]: The loss of adaptation with the dilution of local genes was only found in a small number of individual population pairs in the second and third generations, and for the overall analysis of germination and cumulative fitness in the third generation (chapter 5). In contrast, for some population pairs, significant adaptive value of foreign genes was observed in the admixture analysis (chapter 5). In this case, increasing the proportion of foreign genes resulted in increased hybrid progeny fitness. These results suggest that the loss of local adaptation (and any resulting declines in fitness) is not universal in populations of *R. leptorrhynchoides* and that, in contrast, foreign genes may confer fitness benefits.

7.1.3 Predicting local adaptation and outbreeding depression

Target and Source population size were important predictor variables in both the local adaptation and outbreeding depression experiments. Yet in the outbreeding depression experiment, both Target and Source population size were more consistent in explaining the variability in hybrid progeny fitness between population pairs across a range of fitness traits. For a number of traits across the lifecycle, 20 – 64 % of the variability in hybrid progeny fitness could be explained by log Target and log Source population size, with greatest heterosis in population pairs with small Target and large Source populations. Increased hybrid progeny fitness in small Target populations reflects increased genetic load and bi-parental inbreeding in small populations of *R. leptorrhynchoides*, while greater heterosis in pairs with large Source populations signifies the importance of genetic diversity to hybrid progeny fitness and suggests that drift may play an important role in determining the genetic architecture of small

populations. The benefits of sourcing from large populations was also apparent in the local adaptation experiment, with greatest local adaptation for seedling survivorship and the number of leaves at 12 months in pairs with small Source populations. This negative relationship between local adaptation and Source population size may be due to reduced fitness of inbred individuals from small Source populations, compared to the local Target population, which is reflected as local genotype advantage (local adaptation).

For the majority of fitness traits, geographic and environmental distance between populations had little power to predict patterns of local adaptation or hybrid progeny fitness in the F_1, F_2 and backcross generations in the outbreeding depression experiment, as has been observed in a number of previous studies (e.g. Fenster & Galloway 2000b; Montalvo & Ellstrand 2000; Raabová et al. 2007). However, for above ground biomass and the number of leaves at 12 months, local adaptation was positively related to geographic and environmental distance respectively, suggesting that, for some traits, adaptive differentiation in *R. leptorrhynchoides* may increase with spatial scale and the degree of environmental differentiation between populations. In comparison to the early generations hybrids (i.e. F_1, F_2 and BC's), geographic and environmental distance were able to explain some of the variation in hybrid progeny fitness in the F_3 and backcross generations for the outbreeding depression experiment, although the direction and predictive power of these matrices varied between different fitness traits and generations. The increasing importance of geographic and environmental distance in later generations may reflect the dominance of small population process in determining the fitness outcomes for early generation hybrids, so that the influence of geographic and environmental distance may only become apparent with the declining effects of heterosis in later generations.

7.1.4 Heterosis, self-incompatibility and population size: the two-fold benefit for small populations

The loss of *S*-allele diversity has important implications for mate availability in small populations (Byers & Meagher 1992; Young et al. 2000b), as demonstrated by the significant decrease in fertilisation success with declining population size for diploid and tetraploid populations of *R. leptorrhynchoides* (chapter 6). Furthermore, increased bi-parental inbreeding can have detrimental fitness effects in small populations (Dudash

& Fenster 2000; Heschel & Paige 1995), and as a consequence, small populations may experience greater heterosis following inter-population hybridisation (e.g. Heschel & Paige 1995; Willi & Fischer 2005), as was observed for *R. leptorrhynchoides* (chapter 4). These two processes indicate the potential twofold benefit for small populations following genetic rescue; firstly through the restoration of *S*-allele diversity (chapter 6) and, secondly through heterosis for hybrid progeny fitness (chapter 4). This twofold advantage for small populations has also been demonstrated for *Ranunculus reptans* (Willi et al. 2005), where inter-population crossing increased cross compatibility and resulted in heterosis in F_1 hybrid progeny. These results highlight the importance of population size in determining the outcomes of genetic rescue, and indicate the potential for inter-population hybridisation to increase the long-term viability of small, inbred populations with reduced genetic diversity. Furthermore, this is in contrast to the traditional view that introducing novel genetic diversity constitutes a trade-off with the negative effects of outbreeding depression.

7.2 Management implication for *Rutidosis leptorrhynchoides*

Rutidosis leptorrhynchoides is listed as endangered both at a state and federal level within Australia. Given its high conservation status, and that almost half of the remnant populations of *R. leptorrhynchoides* contain less than 350 individuals, the effective management of small populations is critical to the long-term *in situ* conservation of this species. As discussed above, genetic rescue, through supplementing populations with novel genetic material from larger populations (Ingvarsson 2001; Richards 2000), may improve the viability of small populations through increasing *S*-allele diversity and mate availability (Young et al. 2000b) and/or by counteracting the deleterious effects of inbreeding depression (Dudash & Fenster 2000; Hedrick & Kalinowski 2000). These potential benefits, however, may be offset by the disruption of local adaptation and outbreeding depression following inter-population hybridisation. The results of this thesis have three key implications for the management of small populations of *R. leptorrhynchoides*:

1. Small populations have reduced *S*-allele diversity, which has significant negative consequences for mate availability and fertilisation success (chapter 6). Moreover, greater heterosis observed in small populations (chapter 4) provides

evidence of reduced fitness due to increased bi-parental inbreeding in small populations of this species.

2. Consequently, supplementing small populations with new genetic material is a viable management option for *R. leptorrhynchoides* due to the twofold benefit of augmentation for small populations; Firstly through increased fertilisation success due to the restoration of *S*-allele diversity (chapter 6), and secondly, as a result of increased fitness through heterosis (especially in the F_2 generation) (chapter 4). Furthermore, there was no trade-off with outbreeding depression (chapter 4) or the disruption of local adaptation (chapter 3) in the majority of population pairs. In contrast, superior performance of foreign genotypes in a number of population pairs in the local adaptation experiment (chapter 3) and greater fitness of hybrid progeny with higher levels of admixture (i.e. higher proportion of foreign genes) (chapter 5) indicates the adaptive potential of foreign genotypes in this species.

3. Population size is more important than geographic or environmental distance when choosing source populations for augmentation (chapter 3, 4). Large, genetically diverse source populations should be chosen to ensure maximum heterosis (chapter 4) and to reduce the chance of introducing inbred individuals with lower fitness (chapter 3). However, when deciding between a number of large potential source populations, environmental differentiation and spatial scale should be secondary considerations (chapter 3, 4).

Taken together, these results suggest that augmentation of small populations of *R. leptorrhynchoides* from large, genetically diverse populations is likely to increase population viability through both the restoration of *S*-allele diversity and the fitness benefits of heterosis. Furthermore, the fitness advantage of foreign individuals with novel *S*-alleles should result in rapid penetration of new genes due to negative frequency dependant selection. In the absence of large populations as a seed source for augmentation, mixing collections from several small populations would provide an alternative means of restoring *S*-allele diversity, even though the introduction of genetic

material from small populations is less likely to result in substianital heterosis following inter-population hybridisation.

7.3 Future directions

The aim of this research was to make a contribution to the base of theoretical and applied knowledge regrading the role and significance of local adaptation and outbreeding depression in the management of fragmented plant populations, the genetic mechanisms underlying outbreeding depression and the predictive power of spatial scale, environmental distance and population size. However, there are still many caveats to our knowledge of the importance of outbreeding depression, particularly for species with different life-history traits. In addition, there are still gaps in our understanding of the genetic basis of population divergence and the influence of factors such as mating system, gene flow, selection regimes, genetic drift and ploidy level on the expression of outbreeding depression. Consequently, the following section outlines potential research questions regarding outbreeding depression and *S*-allele diversity that would extend the current knowledge base in a research area that has important implications not only for conservation biology and restoration, but in understanding the evolutionary process of population divergence and speciation. These questions include:

1. Can genetic distance matrices such as Q_{ST} and F_{ST} provide additional insights into patterns of local adaptation and outbreeding depression? In the present study, environmental and geographic distances were used as surrogates of adaptive and evolutionary divergence. However, Q_{ST}, as a direct measure of adaptive differentiation, and F_{ST} which provides information on patterns of coancestry and drift, may have greater power to explain the variability between population pairs in local adaptation and outbreeding depression. Although not included in this thesis, the generation of Q_{ST} and F_{ST} matrices is currently being undertaken for *R. leptorrhynchoides* and will subsequently be used as predictive matrices in the local adaptation and outbreeding depression studies.

2. Although inter-population hybridisation up to the F_3 provides critical insights into the fitness outcomes for early generation hybrids, the fitness of later generation hybrids is important for understanding the implications of inter-

population hybridisation for long-term population viability. In the single plant study to go beyond the F_3 (Erickson & Fenster 2006) superior performance of later generational hybrids and recovery from outbreeding depression was observed for *Chamaecrista fasciculata*. This raises the question of whether increased recombination in later generations would have positive effects on hybrid progeny fitness through the production of advantageous gene combinations, or negative effects through the further loss of co-adaptation.

3. Modelling the fitness effects and dissemination of novel genotypes following augmentation. Modelling can play an important role in examining how *S*-alleles disseminate through the population following augmentation, which has important implications for mean population fitness if foreign genes confer fitness benefits (i.e. heterosis) above the increase in fertilisation success. For example, if genotypes with novel *S*-alleles spread rapidly through the population due to negative frequency dependent selection (as might be expected), then this may influence the spread of heterosis in the generations immediately following augmentation.

4. How does cytological variation (ploidy level) affect the expression of outbreeding depression? As discussed in chapter 1, theoretically, ploidy level may have an important influence on the expression of outbreeding depression due to a number of factors including; a predicted increase in the rate of evolutionary change, the potential for increased gene interactions, alteration of recombination rates, greater population differentiation and reduced inbreeding in polyploid compared to diploid populations (see section 1.3.5.5). An ideal experimental framework to test this hypothesis would be to compare outbreeding depression in a species with a number of different chromosome races, such as *R. leptorrhynchoides*, while controlling for other factors such as environmental and genetic distance that can potentially influence the expression of outbreeding depression.

5. Does mating system influence the level of outbreeding depression? Theory predicts that the magnitude of outbreeding depression should be larger in selfing

species due to reduced recombination, an increase in the likelihood of adaptation to local environmental conditions and greater population differentiation (see chapter 1, section 1.3.5.1). Following these theoretical predictions, further research is required that investigates if there is consistently less outbreeding depression in self-incompatible, compared to self-compatible species. Yet the expectation of greater heterosis following inter-population hyrbidisation in selfing species may counteract outbreeding depression, leading to less outbreeding depression in early generation hybrids. Comparing outbreeding depression in populations with different mating systems within a single species (see Busch 2006), or between closely related species, would provide a relevant assessment of the influence of mating system on the magnitude of outbreeding depression.

6. The role of outbreeding depression in affecting traits that are of importance in mediating co-evolutionary relationships such as (i) soil micro-organisms, (ii) pollinator interactions and (iii) disease, is a research area that has received little empirical investigation. For example, given the importance of co-evolutionary relationships between pathogens and their hosts, further research is required that examines the importance of outbreeding depression in systems where population differentiation for disease resistance is apparent and disease acts as a strong selective agent. Greater outbreeding depression, as a result of increased disease susceptibility in inter-population hybrids (e.g. Goldberg et al. 2005), would indicate the importance of selective agents such as infectious disease to the expression of outbreeding depression and would provide important insights into the genetic mechanisms underlying disease resistance and population divergence.

Theoretical and/or empirical investigations of the above questions would contribute to the knowledge base regarding the importance of outbreeding depression in the management of fragmented populations and the evolutionary implications of population divergence for the transition from micro- to macro-evolution.

APPENDIX

Appendix 2.1: The 27 bioclimatic variables generated from BIOCLIM 3.14 for analysis in the environmental distance matrix.

Variable Number	Bioclimatic Variable	Variable Code
1	Annual Mean Temperature	AnnMeT
2	Temperature Annual Range	TAnnRan
3	Temperature Seasonality (CV*)	TSeas
4	Annual Mean Radiation	AnnMeRad
5	Highest Period of Radiation	HiPerRad
6	Lowest Period of Radiation	LoPerRad
7	Radiation of the Wettest Quarter	RadWetQ
8	Radiation of the Driest Quarter	RadDriQ
9	Radiation of the Warmest Quarter	RadWarQ
10	Radiation of the Coldest Quarter	RadColQ
11	Radiation Seasonality (CV)	RadSeas
12	Isothermality	Iso
13	Mean Diurnal Range	MDR
14	Mean Temperature of Wettest Quarter	MeTWetQ
15	Mean Temperature of Driest Quarter	MeTDriQ
16	Mean Temperature of Warmest Quarter	MeTWarQ
17	Mean Temperature of Coldest Quarter	MeTColQ
18	Maximum Temperature of Warmest Period	MaxTWarP
19	Minimum Temperature of Coldest Period	MinTColP
20	Annual Precipitation	AnnPre
21	Precipitation of the Wettest Quarter	PreWetQ
22	Precipitation of the Driest Quarter	PreDriQ
23	Precipitation of the Warmest Quarter	PreWarQ
24	Precipitation of the Coldest Quarter	PreColQ
25	Precipitation of the Wettest Period	PreWetP
26	Precipitation of the Driest Period	PreDriP
27	Precipitation Seasonality (CV)	PreSeas

Appendix 2.2: The 16 soil variables used in the environmental distance matrix to quantify edaphic variation between sites.

Variable Type and Number	Edaphic Variable	Variable Code
Chemical		
1	Electrical Conductivity (dS/m)	EC
2	Total C (%)	TC
3	Total N (%)	TN
4	Ammonium-Nitrogen (mg/kg)	NH4-N
5	Nitrate-Nitrogen (mg/kg)	NO3-N
6	pH (soil:water)	pH1
7	pH (0.01M CaCl2)	pH2
8	Extractable P (mg/kg)	ExtP
9	Copper (mg/kg)	Cu
10	Iron (mg/kg)	Fe
11	Manganese (mg/kg)	Mn
12	Zinc (mg/kg)	Zn
Composition (soil particle)		
13	Clay (%)	CL
14	Silt (%)	SI
15	Fine Sand (%)	FS
16	Coarse Sand	CS

Appendix 3.1: Summary of the results of the GLM and REML analysis to examine if there were differences between Target (local) and Source (foreign) plants for a range of seedling traits in the first local adaptation experiment including all population pairs (Overall) and for each independent population pair comparison.

Analyses where Origin had a significant effect ($P < 0.05$) on plant performance areighlighted in bold. Population pairs are listed in order of increasing geographic distance between populations. [1]Deviance ratio for GLM analysis (GERM, $SURV_3$, $SURV_6$)

	Population Pair (Geographic distance between populations)																			
	Overall		LW-QB (0.7km)		SR-CC (1.5km)		MA-BA (4.0km)		HH-MA (8.0km)		QB-RH (9.6km)		QB-SR (10.8km)		TR-SA (11.6km)		CR-LW (15.2km)		MJ-CF (27.8km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling																				
Seed weight (SW) (mg)	**16.16**	**<0.001**	2.04	0.153	**13.29**	**<0.001**	**3.88**	**0.049**	1.52	0.217	0.39	0.534	0.07	0.798	1.79	0.181	**8.42**	**0.004**	**11.79**	**<0.001**
Germination (GERM)	0.01	0.932	**5.50**	**0.021**	0.91	0.341	**10.09**	**0.002**	0.00	0.953	**4.40**	**0.038**	0.53	0.470	0.00	0.953	**6.59**	**0.012**	0.12	0.728
Seedling survivorhsip (3 months) ($SURV_3$)	**7.14**	**0.008**	3.41	0.068	0.56	0.455	0.26	0.610	**4.16**	**0.044**	**15.22**	**<.001**	**7.33**	**0.008**	0.08	0.772	1.74	0.190	**14.69**	**<0.001**
Seedling survivorhsip (6 months) ($SURV_6$)	**16.13**	**<0.001**	**6.09**	**0.015**	2.38	0.126	**16.59**	**<.001**	0.00	0.998	1.34	0.250	0.43	0.514	3.42	0.068	0.16	0.686	**5.17**	**0.025**
Number of leaves ($NoLVS_6$)	**14.92**	**<0.001**	0.43	0.511	3.66	0.056	0.07	0.793	**5.60**	**0.018**	**10.57**	**0.001**	**6.54**	**0.011**	0.10	0.755	**9.48**	**0.002**	2.86	0.091
Rosette height ($RosHT_6$) (mm)	1.36	0.244	1.65	0.199	0.70	0.402	1.12	0.291	**10.92**	**<0.001**	**20.85**	**<0.001**	2.55	0.110	**12.22**	**<0.001**	0.32	0.571	0.45	0.504
Leaf size (LFS_6) (mm^2)	0.02	0.882	**4.37**	**0.037**	0.61	0.436	0.44	0.508	0.53	0.467	**13.55**	**<0.001**	**4.70**	**0.030**	**4.80**	**0.028**	**7.68**	**0.006**	1.25	0.263
Index of plant size ($INDPS_6$)	2.93	0.087	1.96	0.162	2.20	0.138	0.19	0.667	2.59	0.107	**18.26**	**<0.001**	0.02	0.894	1.98	0.160	**9.75**	**0.002**	2.14	0.144

	Population Pair (Geographic distance between populations)																	
	RH-CF (34.8km)		CR-CF (37.5km)		MJ-GB (71.9km)		GB-PO (78.9km)		GB-CC (79.9km)		TR-SR (506.2km)		CF-SA (516.0km)		GB-SA (575.1km)		GB-TR (586.2km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling																		
Seed weight (SW) (mg)	**4.95**	**0.026**	**12.48**	**<0.001**	**9.34**	**0.002**	1.17	0.279	1.20	0.273	0.91	0.341	0.10	0.747	0.00	0.967	0.03	0.855
Germination (GERM)	3.71	0.057	1.66	0.201	1.46	0.230	1.71	0.194	0.59	0.443	0.30	0.584	0.07	0.786	0.53	0.468	0.38	0.537
Seedling survivorhsip (3 months) ($SURV_3$)	0.73	0.394	0.44	0.508	**13.08**	**<0.001**	0.01	0.942	0.33	0.569	3.77	0.056	0.07	0.789	2.82	0.096	0.50	0.483
Seedling survivorhsip (6 months) ($SURV_6$)	1.11	0.295	**4.65**	**0.033**	**10.70**	**0.001**	2.68	0.105	0.15	0.702	**8.31**	**0.005**	**31.57**	**<0.001**	0.22	0.643	0.02	0.894
Number of leaves ($NoLVS_6$)	1.63	0.201	2.05	0.152	1.98	0.159	0.45	0.503	0.04	0.847	**11.07**	**<0.001**	0.68	0.410	**11.70**	**<0.001**	3.14	0.076
Rosette height ($RosHT_6$) (mm)	**23.38**	**<0.001**	0.73	0.392	**4.11**	**0.043**	0.71	0.398	**6.02**	**0.014**	**36.50**	**<0.001**	**5.94**	**0.015**	**14.61**	**<0.001**	4.07	0.044
Leaf size (LFS_6) (mm^2)	**21.61**	**<0.001**	1.27	0.259	**4.73**	**0.030**	**4.83**	**0.028**	0.44	0.507	**6.89**	**0.009**	0.09	0.764	2.91	0.088	1.96	0.161
Index of plant size ($INDPS_6$)	**15.17**	**<0.001**	2.66	0.103	2.85	0.091	3.33	0.068	0.33	0.565	**9.56**	**0.002**	0.19	0.665	0.39	0.534	0.20	0.656

Appendix 3.2: Summary of the results of the GLM and REML analysis to examine if there were differences between Target (local) and Source (foreign) plants for a range of traits across the life cycle in the second local adaptation experiment including all population pairs (Overall) and for each independent population pair comparison. Analyses where Origin had a significant effect ($P < 0.05$) on plant performance are highlighted in bold. Population pairs are listed in order of increasing geographic distance between populations. [1]Deviance ratio for GLM analysis (GERM, $SURV_3$, $SURV_6$, $FLOWERST_{12}$, CFI_{24})

	Population Pair (Geographic distance between populations)																			
	Overall		LW-QB (0.7km)		SR-CC (1.5km)		MA-BA (4.0km)		HH-MA (8.0km)		QB-RH (9.6km)		QB-SR (10.8km)		LW-SR (11.4km)		CR-LW (15.2km)		MJ-CF (27.8km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling																				
Seed weight (SW) (mg)	3.43	0.064	0.19	0.659	1.36	0.244	1.35	0.246	1.02	0.313	**10.58**	**0.001**	**5.54**	**0.019**	**10.45**	**0.001**	**18.89**	**<0.001**	1.93	0.164
Germination (GERM)	0.67	0.414	3.58	0.064	**5.86**	**0.019**	2.50	0.120	0.21	0.646	1.97	0.165	0.01	0.927	0.05	0.825	**4.68**	**0.035**	1.74	0.192
Seedling survivorhsip (3 months) (SUR\	**10.53**	**0.001**	2.30	0.141	0.45	0.508	**5.32**	**0.029**	1.39	0.248	0.56	0.459	0.00	0.992	0.59	0.450	0.04	0.843	3.70	0.065
Reproductive adult (12 months)																				
Number of leaves ($NoLVS_{12}$)	1.43	0.231	**15.03**	**<0.001**	**7.50**	**0.006**	**5.30**	**0.021**	1.06	0.303	0.20	0.653	1.18	0.277	3.62	0.057	**8.01**	**0.005**	**9.17**	**0.002**
Plant height (HT_{12}) (mm)	**6.46**	**0.011**	0.12	0.735	3.34	0.068	2.97	0.085	0.00	0.999	**12.49**	**<0.001**	**11.56**	**<0.001**	0.80	0.371	2.28	0.131	**15.92**	**<0.001**
Leaf size (LFS_{12}) (mm^2)	**4.59**	**0.032**	**7.62**	**0.006**	0.00	0.996	3.69	0.055	0.75	0.387	**7.94**	**0.005**	1.21	0.272	**12.06**	**<0.001**	**28.75**	**<0.001**	**25.55**	**<0.001**
Index of plant size ($INDPS_{12}$)	1.72	0.189	0.46	0.497	2.17	0.141	0.49	0.484	1.62	0.203	1.36	0.244	1.93	0.165	0.51	0.475	3.46	0.063	**6.68**	**0.010**
Number of stems ($NoST_{12}$)	0.08	0.784	**10.87**	**<0.001**	0.25	0.616	**7.11**	**0.008**	1.43	0.233	**16.36**	**<0.001**	1.17	0.280	1.89	0.169	**4.63**	**0.032**	**4.08**	**0.043**
Number of flowers and buds ($NoF\&B_{12}$)	0.04	0.839	0.05	0.821	0.99	0.320	0.62	0.433	0.51	0.473	0.09	0.763	0.01	0.911	0.01	0.920	0.03	0.857	1.79	0.181
Flowering Stems ($FLOWERST_{12}$)	**5.38**	**0.021**	1.53	0.222	2.50	0.120	0.27	0.607	0.91	0.345	0.22	0.639	0.02	0.900	0.01	0.913	0.02	0.900	0.51	0.478
Reproductive adult (24 months)																				
Above ground biomass (AGB_{24}) (mg)	**4.01**	**0.045**	**5.12**	**0.024**	**6.24**	**0.012**	0.02	0.892	3.36	0.067	**5.43**	**0.020**	0.62	0.431	0.24	0.623	2.33	0.127	**5.49**	**0.019**
Cumultive fitness index (CFI_{24})	**3.91**	**0.048**	0.00	0.970	0.98	0.326	0.33	0.565	0.39	0.534	0.44	0.508	0.04	0.849	0.07	0.787	0.01	0.911	2.28	0.135

	Population Pair (Geographic distance between populations)																	
	RH-CF (34.8km)		CR-CF (37.5km)		MJ-GB (71.9km)		GB-PO (78.9km)		GB-CC (79.9km)		SR-TR (506.2km)		CF-SA (516.0km)		GB-SA (575.1km)		GB-TR (586.2km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling																		
Seed weight (SW) (mg)	**13.56**	**<0.001**	1.75	0.186	3.38	0.066	0.96	0.328	0.00	0.993	1.19	0.275	0.42	0.515	0.15	0.703	**5.53**	**0.019**
Germination (GERM)	1.59	0.212	1.46	0.232	**9.72**	**0.003**	**7.76**	**0.007**	3.71	0.059	0.12	0.728	0.98	0.326	**5.68**	**0.022**	0.26	0.610
Seedling survivorhsip (3 months) (SUF	0.71	0.406	0.94	0.340	0.82	0.373	**11.20**	**0.002**	0.05	0.828	1.37	0.252	0.78	0.385	2.95	0.101	0.18	0.677
Reproductive adult (12 months)																		
Number of leaves ($NoLVS_{12}$)	**42.83**	**<0.001**	2.37	0.124	**4.39**	**0.036**	**6.95**	**0.008**	0.74	0.389	1.04	0.308	0.01	0.908	0.00	0.953	3.56	0.059
Plant height (HT_{12}) (mm)	1.67	0.197	2.96	0.085	2.58	0.108	**5.03**	**0.025**	3.63	0.057	**37.50**	**<0.001**	0.35	0.552	**5.08**	**0.024**	**9.93**	**0.002**
Leaf size (LFS_{12}) (mm^2)	**49.23**	**<0.001**	**23.61**	**<0.001**	0.57	0.449	0.06	0.814	0.19	0.659	2.37	0.123	7.45	0.006	0.57	0.450	**4.00**	**0.046**
Index of plant size ($INDPS_{12}$)	0.96	0.326	1.53	0.216	0.55	0.460	**4.86**	**0.028**	0.02	0.875	0.65	0.421	0.04	0.838	0.83	0.363	**9.88**	**0.002**
Number of stems ($NoST_{12}$)	**47.04**	**<0.001**	**9.29**	**0.002**	0.30	0.583	**5.54**	**0.019**	3.11	0.078	0.08	0.777	0.03	0.870	2.97	0.085	0.42	0.517
Number of flowers and buds ($NoF\&B_{12}$	0.24	0.627	0.07	0.793	0.43	0.511	**6.66**	**0.010**	0.46	0.497	**16.77**	**<0.001**	1.73	0.188	**5.28**	**0.022**	**9.42**	**0.002**
Flowering Stems ($FLOWERST_{12}$)	7.19	0.010	0.39	0.536	1.88	0.176	2.18	0.145	2.70	0.105	**7.32**	**0.009**	0.10	0.759	**6.24**	**0.017**	1.74	0.193
Reproductive adult (24 months)																		
Above ground biomass (AGB_{24}) (mg)	**3.97**	**0.046**	**4.53**	**0.033**	0.02	0.896	0.81	0.368	2.44	0.119	0.01	0.943	2.53	0.112	**5.44**	**0.020**	0.49	0.486
Cumultive fitness index (CFI_{24})	**6.10**	**0.016**	**4.13**	**0.045**	0.04	0.851	**6.35**	**0.014**	0.13	0.716	2.75	0.101	0.04	0.844	2.04	0.159	**4.13**	**0.046**

Appendix 3.3: A summary of the results of the analysis examining if there is an interaction between the effect of Origin (Target (local) or Source (foreign)) and Experiment for the 16 population pairs common to both local adaptation experiments. Significant model terms ($P < 0.05$) are highlighted in bold.

		Germination		Seedling survivorship	
Population pair	**Factor**	**Dev ratio**	***P* value**	**Dev ratio**	***P* value**
Overall	Origin	0.61	0.435	**21.46**	**<0.001**
	Origin.Experiment	0.08	0.78	**7.57**	**0.006**
LW-QB	Experiment	**14.46**	**<0.001**	**5.84**	**0.017**
	Origin	0.01	0.906	1.31	0.254
	Origin.Experiment	**8.16**	**0.005**	**8.15**	**0.005**
SR-CC	Experiment	**6.06**	**0.015**	**5.1**	**0.025**
	Origin	**4.75**	**0.03**	2.24	0.136
	Origin.Experiment	0.44	0.51	0.44	0.509
MA-BA	Experiment	**42.47**	**<0.001**	**4.38**	**0.038**
	Origin	**7.52**	**0.007**	1.2	0.275
	Origin.Experiment	2.49	0.116	0.11	0.742
HH-MA	Experiment	1.18	0.279	**33.67**	**<0.001**
	Origin	0.04	0.837	**5.29**	**0.023**
	Origin.Experiment	0.07	0.788	**5.09**	**0.025**
QB-RH	Experiment	**16.47**	**<0.001**	**9.28**	**0.003**
	Origin	0.01	0.939	**19.2**	**<0.001**
	Origin.Experiment	**5.44**	**0.021**	2.32	0.13
QB-SR	Experiment	**76.48**	**<0.001**	**10.01**	**0.002**
	Origin	0.11	0.745	**7.87**	**0.006**
	Origin.Experiment	0.13	0.718	3.06	0.082
CR-LW	Experiment	**13.05**	**<0.001**	**34.93**	**<0.001**
	Origin	**10.77**	**0.001**	2.7	0.102
	Origin.Experiment	0.36	0.551	0.38	0.539
MJ-CF	Experiment	2.34	0.128	**18.22**	**<0.001**
	Origin	0.94	0.334	**33.13**	**<0.001**
	Origin.Experiment	1.87	0.173	0.74	0.391
RH-CF	Experiment	**7.07**	**0.008**	**18.09**	**<0.001**
	Origin	0.01	0.912	2.6	0.108
	Origin.Experiment	**5.3**	**0.022**	0.91	0.341
CR-CF	Experiment	**5.55**	**0.019**	**14.25**	**<0.001**
	Origin	0.1	0.753	1.16	0.282
	Origin.Experiment	1.76	0.185	0.1	0.746
MJ-GB	Experiment	**14.38**	**<0.001**	**17.63**	**<0.001**
	Origin	**6.67**	**0.01**	3.23	0.074
	Origin.Experiment	0.38	0.538	**47.92**	**<0.001**
GB-PO	Experiment	**30.2**	**<0.001**	0.49	0.486
	Origin	**11.18**	**<0.001**	0.81	0.368
	Origin.Experiment	2.96	0.087	1.49	0.224
GB-CC	Experiment	**7.16**	**0.008**	0.69	0.406
	Origin	0.39	0.533	0.11	0.74
	Origin.Experiment	**5.11**	**0.025**	0.98	0.324
CF-SA	Experiment	2.16	0.143	**10.13**	**0.002**
	Origin	0.11	0.742	0.42	0.517
	Origin.Experiment	0.31	0.577	0.25	0.616
GB-SA	Experiment	3.65	0.058	**4.41**	**0.037**
	Origin	0.91	0.342	**5.59**	**0.019**
	Origin.Experiment	**6.4**	**0.012**	0.19	0.661
GB-TR	Experiment	0.51	0.476	**22.39**	**<0.001**
	Origin	1.08	0.299	1.03	0.312
	Origin.Experiment	0.14	0.706	1.59	0.209

Appendix 3.4: Repeated measures analysis to examine if the effect of plant Origin on progeny fitness varied between 12 and 24 months across a range of fitness traits. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. Seed weight was included as a covariate in the analysis. Overall analysis includes all population pairs. A significant Origin.Time interaction term identifies variables where there was a significant difference in the effect of plant Origin between 12 and 24 months.

			Population Pair (Geographic distance between populations)																	
	Overall		LW-QB (0.7km)		SR-CC (1.5km)		MA-BA (4.0km)		HH-MA (8.0km)		QB-RH (9.6km)		QB-SR (10.8km)		LW-SR (11.4km)		CR-LW (15.2km)		MJ-CF (27.8km)	
Fitness Trait	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Number leaves (NoLVS)																				
Origin	**6.84**	**0.009**	**13.82**	**<0.001**	**9.10**	**0.003**	**5.40**	**0.020**	0.02	0.893	**4.46**	**0.035**	1.79	0.181	**8.13**	**0.004**	**15.06**	**<0.001**	**9.67**	**0.002**
Time	**25.38**	**<0.001**	1.99	0.158	**55.68**	**<0.001**	0.48	0.490	0.07	0.789	0.79	0.374	2.44	0.119	**10.98**	**<0.001**	**26.87**	**<0.001**	0.22	0.643
Origin.Time	0.97	0.324	3.35	0.067	0.85	0.357	0.75	0.387	**4.42**	**0.036**	2.91	0.088	0.01	0.937	0.05	0.820	2.56	0.110	1.58	0.208
Leaf size (LFS) (mm^2)																				
Origin	**9.54**	**0.002**	**12.54**	**<0.001**	0.25	0.618	1.23	0.267	1.39	0.238	**8.69**	**0.003**	0.03	0.871	**21.06**	**<0.001**	**31.59**	**<0.001**	**36.46**	**<0.001**
Time	**810.30**	**<0.001**	**44.60**	**<0.001**	**29.47**	**<0.001**	**24.51**	**<0.001**	**86.25**	**<0.001**	1.36	0.243	**26.25**	**<0.001**	**67.74**	**<0.001**	**117.94**	**<0.001**	**128.27**	**<0.001**
Origin.Time	0.28	0.595	0.44	0.507	0.38	0.539	**4.83**	**0.028**	0.01	0.905	1.68	0.195	2.26	0.133	0.95	0.329	**6.55**	**0.011**	**9.91**	**0.002**
Height (HT) (mm)																				
Origin	**7.21**	**0.007**	0.05	0.817	**9.66**	**0.002**	0.63	0.429	0.00	0.981	**11.97**	**<0.001**	**5.13**	**0.024**	1.18	0.278	2.18	0.140	**13.32**	**<0.001**
Time	**648.49**	**<0.001**	**56.95**	**<0.001**	**30.26**	**<0.001**	**58.36**	**<0.001**	**47.93**	**<0.001**	**196.03**	**<0.001**	**165.14**	**<0.001**	**31.81**	**<0.001**	1.19	0.276	**88.89**	**<0.001**
Origin.Time	1.01	0.316	0.73	0.393	1.48	0.223	0.70	0.404	0.01	0.911	2.50	0.114	0.94	0.332	0.07	0.798	0.34	0.559	1.14	0.287
Index of plant size (INDPS)																				
Origin	1.67	0.197	0.05	0.831	1.99	0.158	1.29	0.257	0.39	0.534	1.34	0.246	1.00	0.318	1.67	0.196	1.16	0.282	**7.93**	**0.005**
Time	**367.38**	**<0.001**	**41.78**	**<0.001**	0.03	0.856	**19.76**	**<0.001**	**64.07**	**<0.001**	1.99	0.158	**29.51**	**<0.001**	**14.81**	**<0.001**	**8.07**	**0.005**	**81.46**	**<0.001**
Origin.Time	0.06	0.804	1.02	0.313	0.32	0.571	1.10	0.294	**3.99**	**0.046**	3.81	0.051	2.27	0.132	0.71	0.401	3.72	0.054	0.24	0.622
Number of stems (NoST)																				
Origin	1.83	0.176	**10.98**	**<0.001**	3.65	0.056	**4.64**	**0.031**	0.08	0.776	**26.18**	**<0.001**	0.62	0.433	3.66	0.056	**6.38**	**0.012**	**14.36**	**<0.001**
Time	**44.59**	**<0.001**	**13.21**	**<0.001**	**3.96**	**0.047**	0.03	0.857	**7.65**	**0.006**	**12.01**	**<0.001**	1.56	0.212	0.05	0.831	1.11	0.293	**5.15**	**0.023**
Origin.Time	1.48	0.224	3.74	0.053	3.31	0.069	**4.14**	**0.042**	**10.96**	**<0.001**	0.20	0.656	0.80	0.371	0.00	0.957	0.74	0.390	0.54	0.462
Number of flowers and buds (NoF&B)																				
Origin	**4.31**	**0.038**	0.34	0.562	**5.45**	**0.020**	1.33	0.248	0.84	0.360	0.73	0.394	0.15	0.697	0.45	0.502	0.82	0.364	0.50	0.481
Time	**59.45**	**<0.001**	**30.42**	**<0.001**	**53.27**	**<0.001**	**57.17**	**<0.001**	**15.15**	**<0.001**	**79.12**	**<0.001**	**53.45**	**<0.001**	**6.56**	**0.010**	1.71	0.190	**19.55**	**<0.001**
Origin.Time	3.63	0.057	0.23	0.630	**8.12**	**0.004**	3.69	0.055	0.13	0.713	0.88	0.349	0.43	0.511	0.56	0.455	0.08	0.782	1.41	0.234

Appendix 3.4 (continued): Repeated measures analysis to examine if the effect of plant Origin on progeny fitness varied between 12 and 24 months across a range of fitness traits. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. Seed weight was included as a covariate in the analysis. Overall analysis includes all population pairs. A significant Origin.Time interaction term identifies variables where there was a significant difference in the effect of plant Origin between 12 and 24 months.

	Population Pair (Geographic distance between populations)																	
	RH-CF (34.8km)		CR-CF (37.5km)		MJ-GB (71.9km)		GB-PO (78.9km)		GB-CC (79.9km)		SR-TR (506.2km)		CF-SA (516.0km)		GB-SA (575.1km)		GB-TR (586.2km)	
Fitness Trait	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Number leaves (NoLVS)																		
Origin	**50.10**	**<0.001**	**4.78**	**0.029**	**3.93**	**0.047**	3.06	0.080	0.23	0.635	1.33	0.249	0.70	0.403	0.30	0.581	**8.14**	**0.004**
Time	**22.45**	**<0.001**	**139.41**	**<0.001**	1.12	0.290	**43.43**	**<0.001**	**76.41**	**<0.001**	**54.45**	**<0.001**	**106.06**	**<0.001**	**14.65**	**<0.001**	**59.21**	**<0.001**
Origin.Time	1.06	0.304	0.04	0.832	1.45	0.229	**5.77**	**0.016**	**4.07**	**0.044**	0.00	0.944	0.90	0.343	0.32	0.572	0.28	0.595
Leaf size (LFS) (mm^2)																		
Origin	**64.56**	**<0.001**	**33.91**	**<0.001**	0.37	0.545	1.62	0.203	0.05	0.826	2.33	0.127	**10.32**	**0.001**	2.38	0.123	**6.49**	**0.011**
Time	**38.51**	**<0.001**	**49.80**	**<0.001**	**69.10**	**<0.001**	**92.07**	**<0.001**	**86.10**	**<0.001**	**47.22**	**<0.001**	3.65	0.056	**36.78**	**<0.001**	**76.94**	**<0.001**
Origin.Time	0.88	0.348	3.19	0.074	0.46	0.499	2.50	0.114	0.40	0.529	2.15	0.143	0.95	0.329	**4.96**	**0.026**	0.71	0.400
Height (HT) (mm)																		
Origin	**6.05**	**0.014**	**6.83**	**0.009**	**4.96**	**0.026**	0.01	0.939	0.59	0.444	**28.14**	**<0.001**	0.27	0.605	**6.38**	**0.012**	2.14	0.143
Time	**39.94**	**<0.001**	**16.76**	**<0.001**	**66.27**	**<0.001**	**121.96**	**<0.001**	**134.80**	**<0.001**	**39.85**	**<0.001**	0.44	0.508	**43.31**	**<0.001**	**197.10**	**<0.001**
Origin.Time	0.74	0.391	0.01	0.930	0.05	0.816	**9.21**	**0.002**	**5.21**	**0.023**	**12.03**	**<0.001**	0.24	0.622	2.18	0.139	**7.62**	**0.006**
Index of plant size (INDPS)																		
Origin	3.23	0.072	**6.03**	**0.014**	0.80	0.371	**7.08**	**0.008**	0.06	0.810	0.12	0.727	1.39	0.238	0.37	0.544	**17.11**	**<0.001**
Time	1.69	0.194	0.43	0.512	**44.86**	**<0.001**	**170.32**	**<0.001**	**126.49**	**<0.001**	**3.97**	**0.046**	**10.32**	**0.001**	**65.72**	**<0.001**	**143.69**	**<0.001**
Origin.Time	2.28	0.131	0.00	0.951	0.05	0.831	1.04	0.308	0.23	0.629	1.46	0.227	2.90	0.088	**4.94**	**0.026**	1.12	0.289
Number of stems (NoST)																		
Origin	**76.08**	**<0.001**	**10.82**	**0.001**	2.09	0.148	3.60	0.058	**4.23**	**0.040**	0.31	0.575	0.24	0.624	0.78	0.378	2.83	0.093
Time	**17.12**	**<0.001**	**37.91**	**<0.001**	**28.61**	**<0.001**	**35.76**	**<0.001**	**51.39**	**<0.001**	0.61	0.436	**51.49**	**<0.001**	**18.70**	**<0.001**	**58.72**	**<0.001**
Origin.Time	0.03	0.858	1.51	0.220	1.58	0.209	**4.00**	**0.046**	0.30	0.586	0.12	0.731	0.98	0.323	3.72	0.054	0.55	0.459
Number of flowers and buds (NoF&B)																		
Origin	3.15	0.076	0.24	0.625	0.32	0.573	**4.19**	**0.041**	1.39	0.238	**54.72**	**<0.001**	3.02	0.082	0.79	0.375	**19.21**	**<0.001**
Time	1.92	0.166	0.94	0.332	**7.88**	**0.005**	**83.05**	**<0.001**	**57.70**	**<0.001**	**69.79**	**<0.001**	0.54	0.461	**16.18**	**<0.001**	**55.85**	**<0.001**
Origin.Time	**9.14**	**0.002**	0.00	0.960	0.28	0.599	**6.25**	**0.012**	0.02	0.898	**22.49**	**<0.001**	0.02	0.877	3.54	0.060	0.30	0.581

Appendix 4.1: Summary of the results of the REML analysis to examine the effect of Cross Type on progeny fitness for a range of fitness traits across the lifecycle including all population pairs (Overall) and for each independent population pair comparison. Analyses where Cross Type had a significant effect ($P < 0.05$) on progeny fitness are highlighted in bold. Population pairs are listed in order of increasing geographic distance between populations. [1]Leaf size and Index of plant size at 4 months was log transformed prior to analysis

	Population Pair (Geographic distance between populations)													
	Overall		**LW-QB (0.7 km)**		**SR-CC (1.5 km)**		**MA-BA (4.0 km)**		**HH-MA (8.0 km)**		**CR-LW (15.2 km)**		**MJ-CF (27.8 km)**	
Fitness Trait	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Seedling														
Seed weight (SW) (mg)	1.95	0.058	0.75	0.558	1.30	0.245	1.72	0.142	1.06	0.385	1.67	0.153	**3.33**	**0.002**
Juvenile (4 months)														
Number leaves ($NoLVS_4$)	**6.26**	**<0.001**	1.10	0.353	**2.73**	**0.008**	1.77	0.133	1.06	0.384	0.81	0.517	1.29	0.251
Rosette height ($RosHT_4$) (mm)	**7.05**	**<0.001**	0.92	0.453	**5.51**	**<0.001**	0.50	0.735	0.67	0.696	1.57	0.178	**2.43**	**0.017**
Leaf size[1] (LFS_4) (mm^2)	**4.92**	**<0.001**	0.69	0.598	**2.92**	**0.005**	0.25	0.911	1.31	0.239	0.99	0.414	1.90	0.065
Index of plant size[1] ($INDPS_4$)	**5.80**	**<0.001**	0.70	0.594	**4.28**	**<0.001**	1.11	0.348	0.99	0.437	0.93	0.443	**2.38**	**0.020**
Adult (8 months)														
Number leaves ($NoLVS_8$)	1.83	0.076	1.76	0.133	**2.04**	**0.046**	1.16	0.324	1.40	0.202	**2.71**	**0.028**	**2.04**	**0.046**
Plant height (HT_8) (mm)	**3.94**	**<0.001**	0.99	0.411	**2.39**	**0.019**	1.32	0.261	**5.77**	**<0.001**	0.41	0.800	**5.16**	**<0.001**
Leaf size (LFS_8) (mm^2)	1.57	0.140	0.61	0.653	**5.72**	**<0.001**	1.12	0.345	1.51	0.158	**4.25**	**0.002**	0.65	0.712
Index of plant size ($INDPS_8$)	0.86	0.535	1.00	0.406	**2.08**	**0.042**	1.92	0.104	**3.20**	**0.002**	**2.69**	**0.029**	0.88	0.520
Number of stems ($NoST_8$)	1.27	0.263	0.81	0.518	**3.27**	**0.002**	0.44	0.777	0.69	0.679	0.36	0.838	**3.51**	**<0.001**
Reproductive adult (12 months)														
Number leaves ($NoLVS_{12}$)	1.68	0.109	1.00	0.407	**2.90**	**0.005**	1.59	0.173	**5.33**	**<0.001**	**2.76**	**0.026**	**2.21**	**0.031**
Plant height (HT_{12}) (mm)	**2.55**	**0.013**	1.20	0.310	**2.02**	**0.048**	0.29	0.886	**7.06**	**<0.001**	0.48	0.754	**5.34**	**<0.001**
Leaf size (LFS_{12}) (mm^2)	1.56	0.141	0.78	0.537	**3.19**	**0.002**	0.72	0.577	**2.52**	**0.014**	2.33	0.053	1.25	0.276
Number of stems ($NoST_{12}$)	0.80	0.585	1.21	0.302	1.46	0.175	0.53	0.715	0.80	0.589	1.03	0.390	**2.66**	**0.010**
Number of flowers and buds ($NoF\&B_{12}$)	1.52	0.155	0.90	0.464	1.55	0.144	2.12	0.075	**2.04**	**0.046**	**2.70**	**0.029**	0.91	0.500
Above ground biomass (AGB_{12}) (g)	1.72	0.099	0.64	0.637	1.78	0.087	0.25	0.909	1.50	0.164	0.65	0.625	**2.24**	**0.028**
Below ground biomass (BGB_{12}) (g)	1.87	0.069	0.87	0.482	**2.16**	**0.035**	0.38	0.825	1.21	0.293	0.13	0.973	**2.01**	**0.050**
Total biomass (TB_{12}) (g)	1.90	0.065	0.79	0.534	**2.20**	**0.031**	0.09	0.987	1.08	0.375	0.13	0.971	**2.11**	**0.039**

Appendix 4.1 (continued): Summary of the results of the REML analysis to examine the effect of Cross Type on progeny fitness for a range of fitness traits across the lifecycle including all population pairs (Overall) and for each independent population pair comparison.
Analyses where Cross Type had a significant effect ($P < 0.05$) on progeny fitness are highlighted in bold. Population pairs are listed in order of increasing geographic distance between populations. [1]Leaf size and Index of plant size at 4 months was log transformed prior to analysis

	Population Pair (Geographic distance between populations)											
	RH-CF (34.8 km)		**MJ-GB (71.9 km)**		**GB-PO (78.9 km)**		**SR-TR (506.2 km)**		**CF-SA (575.1 km)**		**GB-TR (586.2 km)**	
Variable	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Seedling												
Seed weight (SW) (mg)	**3.42**	**0.008**	0.62	0.648	0.77	0.609	1.34	0.229	1.71	0.145	**3.46**	**0.008**
Juvenile (4 months)												
Number leaves ($NoLVS_4$)	**3.91**	**0.004**	1.14	0.335	**2.25**	**0.027**	1.19	0.306	1.06	0.373	**2.36**	**0.051**
Rosette height ($RosHT_4$) (mm)	**7.83**	**<0.001**	**3.27**	**0.011**	1.46	0.178	1.17	0.319	**4.41**	**0.001**	1.83	0.120
Leaf size (LFS_4) (mm^2)	**8.32**	**<0.001**	1.59	0.173	0.67	0.696	0.60	0.756	0.17	0.952	0.60	0.664
Index of plant size[1] ($INDPS_4$)	**9.19**	**<0.001**	**2.50**	**0.040**	1.71	0.101	0.67	0.700	0.93	0.443	0.85	0.495
Adult (8 months)												
Number leaves ($NoLVS_8$)	**2.82**	**0.024**	**3.45**	**0.008**	**2.95**	**0.004**	0.65	0.715	**2.96**	**0.018**	**3.11**	**0.014**
Plant height (HT_8) (mm)	**5.97**	**<0.001**	**3.88**	**0.004**	1.88	0.069	**3.51**	**<0.001**	0.51	0.730	0.68	0.607
Leaf size (LFS_8) (mm^2)	**2.41**	**0.047**	**15.25**	**<0.001**	**2.41**	**0.018**	**2.07**	**0.043**	2.29	0.057	2.33	0.054
Index of plant size ($INDPS_8$)	0.48	0.750	**3.22**	**0.012**	**3.53**	**<0.001**	1.97	0.055	1.71	0.145	0.57	0.682
Number of stems ($NoST_8$)	**5.09**	**<0.001**	1.44	0.219	1.28	0.255	0.26	0.969	**4.19**	**0.002**	0.18	0.949
Reproductive adult (12 months)												
Number leaves ($NoLVS_{12}$)	**4.33**	**0.002**	**3.45**	**0.008**	0.40	0.900	1.37	0.211	**2.39**	**0.049**	0.57	0.682
Plant height (HT_{12}) (mm)	**3.18**	**0.013**	**3.12**	**0.014**	1.98	0.054	**2.93**	**0.005**	0.69	0.600	0.54	0.706
Leaf size (LFS_{12}) (mm^2)	**2.36**	**0.051**	1.92	0.104	0.26	0.970	1.20	0.298	0.52	0.722	1.24	0.291
Number of stems ($NoST_{12}$)	**7.14**	**<0.001**	**3.22**	**0.012**	0.94	0.475	0.09	0.999	**2.74**	**0.027**	0.21	0.935
Number of flowers & buds ($NoF\&B_{12}$)	1.25	0.286	1.39	0.234	**2.28**	**0.025**	1.41	0.195	1.82	0.121	**5.02**	**<0.001**
Above ground biomass (AGB_{12}) (g)	**2.42**	**0.046**	1.29	0.271	**2.07**	**0.043**	1.79	0.084	0.25	0.910	1.11	0.348
Below ground biomass (BGB_{12}) (g)	**2.60**	**0.034**	2.30	0.057	**2.17**	**0.034**	1.09	0.366	0.44	0.780	**3.14**	**0.014**
Total biomass (TB_{12}) (g)	2.06	0.083	2.33	0.054	**2.41**	**0.018**	0.58	0.773	0.35	0.845	**2.56**	**0.037**

Appendix 4.2: Repeated measures analysis to examine if the effect of Cross Type on progeny fitness varied between the adult (8 months) and reproductive adult (12 month) life stages across a range of fitness traits. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. Seed weight was included as a co-variate in the analysis. Overall analysis includes all population pairs. A significant Cross Type.Time interaction term identifies variables where there was a significant difference in the effect of Cross Type between 8 and 12 months.

	Population Pair (Geographic distance between populations)													
	Overall		LW-QB (0.7 km)		SR-CC (1.5 km)		MA-BA (4.0 km)		HH-MA (8.0 km)		CR-LW (15.2 km)		MJ-CF (27.8 km)	
Fitness Trait	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Number leaves (NoLVS)														
Cross type	1.46	0.138	1.89	0.11	**2.31**	**0.023**	1.38	0.238	**4.39**	**<0.001**	**3.17**	**0.013**	**2.93**	**0.005**
Time	**66.97**	**<0.001**	**10.17**	**0.001**	**6.04**	**0.014**	1.22	0.269	3.17	0.075	**11.34**	**<0.001**	**0.45**	**<0.001**
Cross type.Time	**3.51**	**<0.001**	0.25	0.912	1.43	0.188	0.76	0.554	**2.07**	**0.043**	1.90	0.107	87.63	0.873
Leaf size (LFS) (mm^2)														
Cross type	0.95	0.586	0.28	0.892	**3.85**	**<0.001**	0.44	0.782	0.23	0.979	**5.58**	**<0.001**	1.20	0.296
Time	**639.30**	**<0.001**	**16.37**	**<0.001**	**8.51**	**0.004**	**32.16**	**<0.001**	**95.87**	**<0.001**	**170.46**	**<0.001**	**75.19**	**<0.001**
Cross type.Time	**3.95**	**<0.001**	0.66	0.617	**3.76**	**<0.001**	1.47	0.209	**3.11**	**0.003**	0.97	0.422	0.46	0.866
Height (HT) (mm)														
Cross type	**3.43**	**<0.001**	1.14	0.337	**2.47**	**0.016**	0.40	0.812	**6.91**	**<0.001**	0.63	0.643	**5.71**	**<0.001**
Time	**268.64**	**<0.001**	**157.07**	**<0.001**	1.80	0.18	**25.22**	**<0.001**	**15.90**	**<0.001**	**22.42**	**<0.001**	**9.61**	**0.002**
Cross type.Time	**3.45**	**0.001**	0.59	0.672	0.40	0.905	**5.53**	**<0.001**	0.51	0.824	0.35	0.843	0.31	0.952
Index of plant size (INDPS)														
Cross type	0.61	0.747	0.89	0.466	1.01	0.424	1.85	0.116	**2.91**	**0.005**	1.34	0.253	0.70	0.671
Time	**740.74**	**<0.001**	**17.74**	**<0.001**	0.01	0.907	**16.95**	**<0.001**	**112.65**	**<0.001**	**47.99**	**<0.001**	**227.17**	**<0.001**
Cross type.Time	0.74	0.638	0.72	0.578	1.77	0.088	1.70	0.146	1.02	0.418	1.23	0.298	0.47	0.855
Number of stems (NoST)														
Cross type	1.02	0.417	1.00	0.407	**2.36**	**0.021**	0.51	0.728	0.77	0.613	0.62	0.647	**3.41**	**0.001**
Time	**132.18**	**<0.001**	**21.73**	**<0.001**	**36.01**	**<0.001**	0.07	0.796	**45.04**	**<0.001**	2.96	0.086	**67.39**	**<0.001**
Cross type.Time	**2.55**	**0.013**	0.78	0.536	**2.91**	**0.005**	0.26	0.905	0.75	0.631	1.30	0.269	0.71	0.66

Appendix 4.2 (continued): Results of the repeated measures analysis to examine if the effect of Cross Type on progeny fitness varied between the adult (8 months) and reproductive adult (12 month) life stages across a range of fitness traits. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. Seed weight was included as a co-variate in the analysis. Overall analysis includes all population pairs. A significant Cross Type.Time interaction term identifies variables where there was a significant difference in the effect of Cross Type between 8 and 12 months.

	Population Pair (Geographic distance between populations)											
	RH-CF (34.8 km)		MJ-GB (71.9 km)		GB-PO (78.9 km)		SR-TR (506.2 km)		CF-SA (575.1 km)		GB-TR (586.2 km)	
Fitness Trait	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value	Wald/d.f.	*P* value
Number leaves (NoLVS)												
Cross type	**5.22**	**<0.001**	**4.52**	**0.001**	1.40	0.201	1.09	0.365	**3.10**	**0.015**	1.10	0.356
Time	**18.87**	**<0.001**	0.01	0.941	**39.37**	**<0.001**	**24.76**	**<0.001**	1.08	0.827	**70.96**	**<0.001**
Cross type.Time	0.65	0.626	0.73	0.572	1.30	0.248	1.12	0.345	0.05	0.363	**3.22**	**0.012**
Leaf size (LFS) (mm^2)												
Cross type	**3.60**	**0.006**	**13.35**	**<0.001**	1.69	0.106	1.92	0.062	1.73	0.141	1.17	0.323
Time	**32.08**	**<0.001**	**127.93**	**<0.001**	**319.57**	**<0.001**	0.00	0.974	**66.11**	**<0.001**	**287.32**	**<0.001**
Cross type.Time	0.11	0.978	**8.27**	**<0.001**	1.33	0.23	0.79	0.595	2.02	0.089	**2.61**	**0.034**
Height (HT) (mm)												
Cross type	**5.36**	**<0.001**	**3.85**	**0.004**	**2.06**	**0.044**	**3.39**	**0.001**	0.62	0.648	0.50	0.739
Time	**36.14**	**<0.001**	**9.23**	**0.002**	**57.37**	**<0.001**	**5.29**	**0.021**	**72.18**	**<0.001**	**23.83**	**<0.001**
Cross type.Time	1.63	0.164	1.29	0.27	0.72	0.652	0.34	0.935	2.30	0.056	1.31	0.263
Index of plant size (INDPS)												
Cross type	0.51	0.73	1.16	0.326	**2.72**	**0.008**	1.61	0.128	1.41	0.229	0.82	0.509
Time	**0.18**	**<0.001**	**92.52**	**<0.001**	**377.58**	**<0.001**	**36.17**	**<0.001**	**51.39**	**<0.001**	**362.91**	**<0.001**
Cross type.Time	68.95	0.95	**3.91**	**0.004**	**2.42**	**0.018**	1.01	0.423	0.40	0.811	0.21	0.934
Number of stems (NoST)												
Cross type	**4.79**	**<0.001**	2.28	0.058	1.08	0.373	0.16	0.993	**3.71**	**0.005**	0.12	0.976
Time	**32.68**	**<0.001**	0.85	0.356	**35.98**	**<0.001**	**16.82**	**<0.001**	0.02	0.901	**24.90**	**<0.001**
Cross type.Time	1.13	0.341	1.45	0.214	1.64	0.119	0.58	0.77	1.25	0.288	0.39	0.816

Appendix 4.3: Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control) for seedling germination and survivorship including all population pairs (Overall) and for the each independent population pair comparison. Positive values are where mean germinaton and survivorship in each crossing treatment (F_1, F_2, F_3 and BC's) was greater than the control and represent the % increase in GERM or $SURV_3$. Negative values are where mean germination and survivorship in each crossing treatment was less than the control and represent the % decrease in GERM or SURV3. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$). Population pairs are listed in order of increasing geographic distance between populations.

[1]For each population pair, seedlings in each maternal line were pooled for this analysis so that values represent family means for each crossing treatment. *For MJ-CF the overall GLM was non-significant but the individual *P*-value for the comparison between the Control and BC_{TARGET} (F_2 *x* Target) was significant ($P = 0.017$)

		Population Pair											
Fitness trait	**Overall**	**LW-QB**	**SR-CC**	**MA-BA**	**HH-MA**	**CR-LW**	**MJ-CF**	**RH-CF**	**MJ-GB**	**GB-PO**	**SR-TR**	**CF-SA**	**GB-TR**
Seedling													
Germination (GERM)													
P value	**<0.001**	0.623	**0.009**	0.390	**0.029**	0.433	0.117	**0.001**	**0.009**	**<0.001**	**<0.001**	**0.008**	0.330
F_1	1.7	-0.9	1.6	9.7	0.6	2.0	7.2	-1.2	3.9	-3.8	**-13.2**	12.0	2.2
BC (F_1 x Source)	**6.0**	4.9	10.6	10.4	-9.1	-5.1	-0.6	**18.9**	**16.6**	-10.6	9.3	**19.2**	6.0
F_2 (F_1 x F_1)	**6.8**	5.6	1.5	10.5	-8.1	6.9	7.3	**17.8**	**17.7**	**-19.4**	**12.7**	**28.6**	5.1
BC (F_1 x Target)	**9.1**	5.2	**14.7**	10.4	2.3	3.5	11.2	**16.9**	**21.9**	**11.1**	3.0	**19.5**	-4.7
BC (F_2 x Source)	**11.1**		-4.4		10.8		6.0			**12.6**	2.7		
F_3 (F_2 x F_2)	**16.4**		**18.2**		10.8		8.8			9.8	9.7		
BC (F_2 x Target)	**18.5**		9.2		**15.7**		**19.1***			**12.1**	**18.7**		
Survivorship[1] ($SURV_3$)													
P value	**0.027**	**0.012**	0.391	0.288	0.060	0.218	**0.008**	0.584	0.856	0.503	0.783	0.991	0.849
F_1	1.0	2.5	2.9	6.6	0.1	-1.1	-1.2	2.5	-1.1	0.9	2.3	-0.3	0.0
BC (F_1 x Source)	0.3	3.1	-0.4	2.3	-5.5	-2.9	2.8	0.5	1.8	-0.3	0.5	0.9	0.9
F_2 (F_1 x F_1)	0.5	**5.9**	1.9	6.3	-0.8	-6.0	3.2	-3.5	0.9	-2.3	-0.3	0.3	0.0
BC (F_1 x Target)	-0.2	**7.5**	1.4	1.8	-4.0	-4.5	-3.3	1.3	-3.8	0.5	0.4	0.0	1.6
BC (F_2 x Source)	1.5		3.9		-7.7		**5.8**			1.1	1.7		
F_3 (F_2 x F_2)	2.7		0.8		2.8		4.1			-0.2	3.3		
BC (F_2 x Target)	**3.9**		2.2		1.4		**5.8**			2.8	3.5		

Appendix 4.4: Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control, as a % of Control) for the mean number of leaves and mean rosette height at the juvenile life stage (4 months) including all population pairs (Overall) and for the each independent population pair comparison. Positive values are where the mean of each fitness trait in the crossing treatment (F_1, F_2, F_3 and BC's) was greater than the control and represents the % increase in plant growth. Negative values are where the mean of each fitness trait in the crossing treatment was less than the control and represents the % decrease in plant growth. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$). Population pairs are listed in order of increasing geographic distance between populations.

		Population Pair											
Fitness trait	**Overall**	**LW-QB**	**SR-CC**	**MA-BA**	**HH-MA**	**CR-LW**	**MJ-CF**	**RH-CF**	**MJ-GB**	**GB-PO**	**SR-TR**	**CF-SA**	**GB-TR**
Juvenile (4 months)													
Number leaves ($NoLVS_4$)													
P value	**<0.001**	0.353	**0.008**	0.133	0.384	0.517	0.422	**0.004**	0.335	**0.027**	0.306	0.373	0.051
F_1	-0.8	-1.0	**-20.5**	-4.9	-2.6	-7.3	16.4	5.9	10.5	12.0	4.5	-0.2	-14.1
BC (F_1 x Source)	**11.7**	-2.1	-0.5	4.2	3.1	-3.7	20.1	**38.0**	23.7	15.0	13.1	-9.6	4.1
F_2 (F_1 x F_1)	**15.0**	10.7	6.8	3.7	5.5	-1.0	20.1	**42.8**	20.4	**24.5**	11.3	-3.9	8.3
BC (F_1 x Target)	**11.0**	2.1	5.8	-6.5	14.9	3.1	24.2	**33.9**	8.2	**27.4**	2.8	-3.9	-11.3
BC (F_2 x Source)	5.3		-4.0		11.2		5.7			21.4	1.8		
F_3 (F_2 x F_2)	**18.0**		14.6		15.9		13.5			**34.3**	8.0		
BC (F_2 x Target)	0.6		-7.4		-3.3		18.5			**37.6**	-13.3		
Rosette height ($RosHT_4$)													
P value	**<0.001**	0.453	**<0.001**	0.735	0.696	0.178	**0.017**	**<0.001**	**0.011**	0.178	0.319	**0.001**	0.120
F_1	**5.2**	-2.6	**-9.7**	8.6	2.7	-8.9	**19.1**	**14.8**	11.6	3.4	10.6	**21.0**	-4.0
BC (F_1 x Source)	**11.5**	6.1	-1.2	7.3	0.7	-13.7	**23.0**	**34.1**	**22.2**	-3.3	15.1	**16.6**	15.9
F_2 (F_1 x F_1)	**13.3**	10.4	**11.5**	9.2	10.8	-12.7	**21.4**	**34.9**	15.9	14.5	6.6	**19.8**	4.5
BC (F_1 x Target)	**9.6**	2.7	**15.2**	4.4	5.6	-3.9	**25.1**	**29.6**	-2.3	10.6	9.6	7.0	-3.1
BC (F_2 x Source)	0.4		-8.8		6.3		4.2			-7.9	6.7		
F_3 (F_2 x F_2)	4.0		0.2		7.2		10.3			-4.6	5.3		
BC (F_2 x Target)	6.3		0.5		3.8		**22.4**			14.6	-1.3		

Appendix 4.5: Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control, as % of Control) for number of leaves and plant height at the adult life stage (8 months) including all population pairs (Overall) and for the each independent population pair comparison. Positive values are where the mean of each fitness trait in the crossing treatment (F_1, F_2, F_3 and BC's) was greater than the control and represents the % increase in plant growth. Negative values are where the mean of each fitness trait in the crossing treatment was less than the control and represents the % decrease in plant growth. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$). Population pairs are listed in order of increasing geographic distance between populations.

		Population Pair											
Fitness trait	**Overall**	**LW-QB**	**SR-CC**	**MA-BA**	**HH-MA**	**CR-LW**	**MJ-CF**	**RH-CF**	**MJ-GB**	**GB-PO**	**SR-TR**	**CF-SA**	**GB-TR**
Adult (8 months)													
Number leaves ($NoLVS_8$)													
P value	0.076	0.133	**0.046**	0.324	0.202	**0.028**	**0.046**	**0.024**	**0.008**	**0.004**	0.715	**0.018**	**0.014**
F_1	2.7	65.7	-0.4	16.9	-5.4	-9.5	-2.3	-6.3	**19.21**	**20.6**	-5.1	37.9	-0.9
BC (F_1 x Source)	4.7	25.6	7.8	25.3	-11.8	-8.0	-9.0	**-11.0**	**21.56**	**18.2**	-1.03	**56.8**	18.5
F_2 (F_1 x F_1)	7.4	25.1	9.4	40.0	-3.9	**-21.8**	8.2	1.4	**25.56**	**19.0**	0.62	**53.7**	8.1
BC (F_1 x Target)	3.8	24.8	-2.3	16.8	-1.3	-1.3	7.3	4.2	**18.44**	11.7	-4.96	22.5	-2.4
BC (F_2 x Source)	7.3		11.2		-3.2		1.0			8.7	1.31		
F_3 (F_2 x F_2)	3.0		**14.7**		-9.8		6.5			-1.5	-8.21		
BC (F_2 x Target)	1.5		4.8		-8.8		-3.5			14.5	-5.17		
Plant height (HT_8)													
P value	**<0.001**	0.411	**0.019**	0.261	**<0.001**	0.800	**<0.001**	**<0.001**	**0.004**	0.069	**<0.001**	0.730	0.607
F_1	**7.7**	18.7	4.9	7.3	**-10.6**	-3.5	**27.5**	**22.1**	**24.5**	13.8	-1.9	6.1	2.5
BC (F_1 x Source)	**5.8**	39.9	-0.5	20.4	**-21.3**	-2.8	**35.7**	**23.0**	10.1	17.4	**-11.5**	-3.7	-2.6
F_2 (F_1 x F_1)	**6.9**	33.9	-1.4	26.8	**-8.7**	-2.2	**17.8**	**14.0**	10.6	18.9	-4.3	12.7	-3.3
BC (F_1 x Target)	**6.5**	20.1	6.1	8.2	**-8.1**	3.8	12.3	**17.6**	5.5	10.6	3.4	5.8	4.3
BC (F_2 x Source)	1.6		-7.9		**-20.4**		**25.8**			8.8	-3.8		
F_3 (F_2 x F_2)	-1.9		-2.7		**-17.9**		8.3			2.9	-3.3		
BC (F_2 x Target)	1.1		-2.2		**-13.7**		9.6			19.1	**-8.4**		

Appendix 4.6: Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control, as % of Control) for the number of stems and total biomass at the reproductive adult life stage (12 months) including all population pairs (Overall) and for each independent population pair comparison. Positive values are where the mean of each fitness trait in the crossing treatment (F_1, F_2, F_3 and BC's) was greater than the Control and represents the % increase in plant growth. Negative values are where the mean of each fitness trait in the crossing treatment was less than the Control and represents the % decrease in plant growth. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$). Population pairs are listed in order of increasing geographic distance between populations.

Fitness Trait	Overall	LW-QB	SR-CC	MA-BA	HH-MA	CR-LW	MJ-CF	RH-CF	MJ-GB	GB-PO	SR-TR	CF-SA	GB-TR
Reproductive adult (12 months)													
Number of stems ($NoST_{12}$)													
P value	0.585	0.302	0.175	0.715	0.589	0.390	**0.010**	**<0.001**	**0.012**	0.475	0.999	**0.027**	0.935
F_1	0.9	54.8	-2.7	-8.4	7.7	-11.1	-10.7	**17.1**	**20.8**	17.3	2.1	29.4	-5.5
BC_{SOURCE} (F_1 x Source)	0.2	34.9	2.4	0.1	-2.9	-9.7	**-19.1**	**-24.6**	**22.3**	4.6	2.2	**63.1**	1.4
F_2 (F_1 x F_1)	2.8	23.8	2.8	15.7	1.6	-7.1	-4.1	**-13.8**	**27.6**	6.6	0.3	38.4	-1.0
BC_{TARGET} (F_1 x Target)	1.6	32.3	-7.5	-1.6	5.6	-1.9	-0.7	-0.1	15.1	11.6	-0.4	15.6	-0.9
BC_{SOURCE} (F_2 x Source)	7.5		7.0		10.9		-6.7			11.2	3.8		
F_3 (F_2 x F_2)	5.9		7.9		6.3		2.9			-2.2	0.2		
BC_{TARGET} (F_2 x Target)	2.7		-6.2		0.5		1.4			6.1	2.3		
Total biomass (TB_{12})													
P value	0.065	0.534	**0.031**	0.987	0.375	0.971	**0.039**	0.083	0.054	**0.018**	0.773	0.845	**0.037**
F_1	2.1	32.3	**-7.8**	-1.7	2.0	-3.7	**12.7**	-2.3	6.9	8.8	0.1	3.2	-8.1
BC_{SOURCE} (F_1 x Source)	-0.7	10.6	**-9.7**	0.9	-5.6	-1.5	4.8	1.6	9.8	0.5	-5.0	-0.1	-6.2
F_2 (F_1 x F_1)	4.4	20.4	**-7.3**	4.7	4.2	-0.9	**14.5**	7.9	3.9	**11.3**	-0.9	6.2	4.1
BC_{TARGET} (F_1 x Target)	2.1	13.7	**-8.2**	1.5	4.7	0.6	**15.7**	5.3	-3.2	7.0	1.9	-5.1	-4.8
BC_{SOURCE} (F_2 x Source)	-1.0		-8.0		1.0		-1.0			7.0	-1.2		
F_3 (F_2 x F_2)	0.3		-0.9		0.6		5.8			-8.7	-1.9		
BC_{TARGET} (F_2 x Target)	1.0		**-9.0**		-1.3		9.7			8.4	-1.2		

Appendix 4.6 (continued): Percentage difference between the Control (within Target population cross) and each crossing treatment (Treatment - Control, as a % of the Control) for a number of fitness traits at the reproductive adult life stage (12 months) including all population pairs (Overall) and for each independent population pair comparison. Positive values are where the mean of each fitness trait in the crossing treatment (F_1, F_2, F_3 and BC's) was greater than the Control and represents the % increase in plant growth and/or reproduction. Negative values are where the mean of each fitness trait in the crossing treatment was less than the Control and represents the % decrease in plant growth and/or reproduction. Positive values in bold indicate significant heterosis ($P < 0.05$) and negative values in bold indicate significant outbreeding depression ($P < 0.05$). Population pairs are listed in order of increasing geographic distance between populations. *Analyses where the overall GLM was non-significant, but individual model terms for comparisons between the Control and other crossing treatments were significant ($P < 0.05$).

		Population Pair											
Fitness Trait	**Overall**	**LW-QB**	**SR-CC**	**MA-BA**	**HH-MA**	**CR-LW**	**MJ-CF**	**RH-CF**	**MJ-GB**	**GB-PO**	**SR-TR**	**CF-SA**	**GB-TR**
Reproductive adult (12 months)													
Number of flowers and buds ($NoF\&B_{12}$)													
P value	0.155	0.464	0.144	0.075	**0.046**	**0.029**	0.500	0.286	0.234	**0.025**	0.195	0.121	**<0.001**
F_1	8.4	16.9	15.7	38.6	**-29.0**	-5.5	-18.7	3.0	19.6	7.1	12.5	75.1	**90.5**
BC_{SOURCE} (F_1 x Source)	11.9	51.7	23.4	30.1	**-28.5**	-8.3	-34.0	-22.3	21.1	**60.2**	39.2	27.9	**97.9**
F_2 (F_1 x F_1)	11.7	29.3	38.1	22.4	-13.8	8.3	-21.6	-11.4	3.2	6.0	30.4	53.1	**58.1**
BC_{TARGET} (F_1 x Target)	6.1	-0.5	31.6	-3.6	-13.9	**33.9**	-14.5	-3.3	-9.9	-5.7	11.2	15.4	**60.4**
BC_{SOURCE} (F_2 x Source)	-5.0		-0.2		**-30.0**		-16.0			-9.0	18.8		
F_3 (F_2 x F_2)	3.0		40.6		**-31.3**		-16.0			9.6	11.7		
BC_{TARGET} (F_2 x Target)	-0.2		26.9		**-27.3**		-7.5			13.2	3.2		
Number of flowering stems ($FLOWERST_{12}$)													
P value	0.734	0.676	0.486	0.085	0.122	**0.015**	0.359	0.537	0.456	0.651	0.862	0.806	**0.018**
F_1	3.8	-9.3	22.3	**50.6***	**-26.2***	7.5	-13.9	-2.0	-1.9	-11.9	-0.9	0.9	**62.9**
BC_{SOURCE} (F_1 x Source)	6.8	19.3	25.6	**50.7***	**-32.1***	18.2	-17.4	-3.1	9.9	15.8	15.8	-22.7	**56.8**
F_2 (F_1 x F_1)	3.1	19.1	25.3	9.7	-11.0	12.9	**-30.9***	-2.0	-13.8	-10.6	13.8	-11.2	**61.1**
BC_{TARGET} (F_1 x Target)	0.2	-9.2	28.5	5.2	-23.4	**35.7**	-18.7	-20.1	-16.3	-17.5	1.4	-8.5	**65.0**
BC_{SOURCE} (F_2 x Source)	-5.4		2.7		-7.7		-31.4			-16.0	2.8		
F_3 (F_2 x F_2)	2.0		25.1		**-33.4***		-6.9			14.1	8.3		
BC_{TARGET} (F_2 x Target)	3.1		38.2		-17.5		-22.3			3.5	-1.2		
Cumulative fitness index (CFI_{12})													
P value	**<0.001**	0.748	**0.015**	0.066	0.617	0.512	0.806	0.304	0.168	0.768	0.079	0.394	**0.035**
F_1	11.0	17.5	20.5	43.0	-17.6	-5.2	1.1	-0.8	17.3	0.3	-3.3	47.5	44.4
BC_{SOURCE} (F_1 x Source)	**20.8**	19.7	17.9	**55.2**	-21.3	-2.1	-0.3	35.0	**64.5**	14.9	27.9	-0.4	**49.5**
F_2 (F_1 x F_1)	**23.1**	47.6	20.8	22.5	3.0	8.6	-19.3	23.0	22.0	-8.9	**53.3**	33.3	**79.6**
BC_{TARGET} (F_1 x Target)	**24.0**	30.5	**56.4**	19.7	2.3	26.5	4.0	29.5	42.1	13.3	28.7	6.1	34.7
BC_{SOURCE} (F_2 x Source)	8.0		-13.3		12.5		-21.8			-1.5	18.9		
F_3 (F_2 x F_2)	**21.5**		**46.7**		-8.0		-0.7			3.2	23.3		
BC_{TARGET} (F_2 x Target)	**32.4**		**45.6**		-1.2		3.7			38.8	32.3		

Appendix 5.1: The relative importance of admixture and recombination for seedling germination and juvenile growth ($NoLVS_4$ and $RosHT_4$) for the 5 population pairs of Rutidosis leptorrhynchoides with F_2 and F_3 progeny. A significant Admixture.Recombination term indicates an interaction between the effects of these two genetic mechanisms on hybrid progeny fitness. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. [1]Deviance ratio for GLM analysis (GERM).

	Population Pair (Geographic distance between populations)											
	Overall		SR-CC (1.5 km)		HH-MA (8.0 km)		MJ-CF (27.8 km)		GB-PO (78.9 km)		SR-TR (506.2 km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling												
Germination (GERM)												
Admixture	**9.00**	**<0.001**	1.68	0.190	2.99	0.053	**4.00**	**0.020**	**11.87**	**<0.001**	2.85	0.061
Recombination	**14.67**	**<0.001**	0.01	0.911	**5.65**	**0.019**	0.33	0.566	**17.63**	**<0.001**	2.22	0.138
Admixture.Recombination	1.40	0.248	**6.64**	**0.002**	0.44	0.643	0.39	0.680	**3.50**	**0.032**	**3.26**	**0.041**
Juvenile (4 months)												
Number leaves ($NoLVS_4$)												
Admixture	1.82	0.162	2.25	0.106	0.10	0.906	0.65	0.521	0.42	0.655	0.37	0.694
Recombination	**4.61**	**0.032**	2.30	0.129	0.80	0.370	1.66	0.198	0.32	0.572	2.46	0.117
Admixture.Recombination	1.78	0.168	1.00	0.368	2.68	0.069	0.01	0.990	0.30	0.742	0.53	0.588
Rosette height ($RosHT_4$)												
Admixture	**4.39**	**0.012**	**9.40**	**<0.001**	1.00	0.367	1.00	0.369	**3.53**	**0.029**	2.86	0.057
Recombination	**16.80**	**<0.001**	**15.83**	**<0.001**	0.86	0.355	**4.33**	**0.038**	1.40	0.237	1.07	0.300
Admixture.Recombination	0.11	0.892	0.44	0.642	1.04	0.353	0.75	0.472	1.17	0.309	0.42	0.660

Appendix 5.2: The relative importance of admixture and recombination for adult growth, reproduction and cumulative fitness for the 5 population pairs of Rutidosis leptorrhynchoides with F_2 and F_3 progeny. A significant Admixture.Recombination term indicates an interaction between the effects of these two genetic mechanisms on hybrid progeny fitness. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. [1]Deviance ratio for GLM analysis (Cumulative Fitness).

	Population Pair (Geographic distance between populations)											
	Overall		SR-CC (1.5 km)		HH-MA (8.0 km)		MJ-CF (27.8 km)		GB-PO (78.9 km)		SR-TR (506.2 km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Adult (8 months)												
Number leaves ($NoLVS_8$)												
Admixture	0.89	0.412	**3.82**	**0.022**	0.68	0.509	**4.06**	**0.017**	0.92	0.400	0.24	0.783
Recombination	1.43	0.233	2.06	0.151	1.14	0.286	0.01	0.910	**5.53**	**0.019**	1.38	0.240
Admixture.Recombination	2.49	0.083	0.11	0.896	2.35	0.096	2.51	0.082	0.59	0.553	0.91	0.404
Plant height (HT_8)												
Admixture	0.86	0.422	**3.34**	**0.035**	**5.54**	**0.004**	**6.20**	**0.002**	0.17	0.840	**3.36**	**0.035**
Recombination	**12.36**	**<0.001**	**8.15**	**0.004**	**6.97**	**0.008**	3.22	0.073	0.51	0.475	0.00	0.998
Admixture.Recombination	0.68	0.505	0.73	0.484	1.54	0.215	0.50	0.609	2.26	0.104	**5.75**	**0.003**
Reproductive adult (12 months)												
Total biomass (TB_{12})												
Admixture	**6.55**	**0.001**	1.18	0.308	2.54	0.079	2.83	0.059	1.20	0.301	1.57	0.208
Recombination	2.35	0.125	0.66	0.416	0.07	0.787	**3.94**	**0.047**	2.71	0.100	0.14	0.708
Admixture.Recombination	1.57	0.208	0.90	0.405	2.70	0.067	0.07	0.937	**3.89**	**0.020**	0.59	0.556
Number of flowers and buds ($NoF\&B_{12}$)												
Admixture	0.43	0.650	0.49	0.613	0.81	0.445	0.98	0.376	**3.01**	**0.049**	1.72	0.180
Recombination	3.28	0.070	0.24	0.628	2.67	0.102	0.73	0.394	0.69	0.407	0.74	0.389
Admixture.Recombination	0.55	0.579	1.34	0.263	0.46	0.633	0.20	0.818	**3.81**	**0.022**	0.20	0.818
Cumulative Fitness Index (CFI_{12})												
Admixture	**3.45**	**0.032**	**4.72**	**0.010**	0.98	0.378	0.92	0.400	1.23	0.295	1.18	0.310
Recombination	0.06	0.805	0.02	0.894	0.13	0.722	0.02	0.884	0.36	0.551	0.03	0.868
Admixture.Recombination	0.23	0.794	1.88	0.156	3.09	**0.048**	1.02	0.364	0.76	0.470	0.96	0.384

Appendix 5.3.: The effect of cytoplasmic background (home or foreign) on a number of fitness traits across the lifecycle for F_1 progeny including all population pairs (overall analysis) and for each individual population pair comparison. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. [1]Deviance ratio for GLM analysis (GERM and CFI_{12})

	Overall		LW-QB (0.7 km)		SR-CC (1.5 km)		MA-BA (4.0 km)		HH-MA (8.0 km)		CR-LW (15.2 km)		MJ-CF (27.8 km)	
Fitness Trait	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling														
Germination (GERM)	1.54	0.216	2.37	0.131	**5.32**	**0.026**	3.11	0.085	0.26	0.614	0.04	0.839	3.99	0.052
Juvenile (4 months)														
Number leaves ($NoLVS_4$)	0.02	0.875	2.49	0.114	0.89	0.345	0.97	0.324	0.73	0.394	0.97	0.324	1.05	0.305
Rosette height ($RosHT_4$) (mm)	0.00	0.970	0.41	0.520	0.34	0.561	2.55	0.111	0.04	0.836	1.01	0.315	0.14	0.713
Adult (8 months)														
Number leaves ($NoLVS_8$)	0.02	0.887	0.30	0.581	0.04	0.849	0.15	0.701	0.61	0.435	1.05	0.306	0.49	0.486
Plant height (HT_8) (mm)	0.28	0.598	0.09	0.764	1.86	0.173	0.14	0.704	0.09	0.763	0.36	0.549	**4.17**	**0.041**
Reproductive adult (12 months)														
Number of flowers and buds ($NoF\&B_{12}$)	3.31	0.069	0.01	0.936	0.99	0.321	0.13	0.723	0.13	0.716	0.01	0.934	**4.30**	**0.038**
Total biomass (TB_{12}) (g)	0.27	0.604	0.02	0.885	0.59	0.444	0.14	0.710	0.11	0.737	0.48	0.489	1.03	0.309
Cumulative fitness index (CFI_{12})	0.26	0.608	0.28	0.601	0.36	0.553	0.72	0.400	0.40	0.532	0.00	0.958	0.15	0.698

Appendix 5.3 (continued): The effect of cytoplasmic background (home or foreign) on a number of fitness traits across the lifecycle for F_1 progeny including all population pairs (overall analysis) and for each individual population pair comparison. Population pairs are listed in order of increasing geographic distance between populations. Significant model terms ($P < 0.05$) are highlighted in bold. [1]Deviance ratio for GLM analysis (GERM and CFI_{12})

	RH-CF (34.8 km)		MJ-GB (71.9 km)		GB-PO (78.9 km)		SR-TR (506.2 km)		CF-SA (575.1 km)		GB-TR (586.2 km)	
Variable	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value	Wald/d.f.[1]	*P* value
Seedling												
Germination (GERM)	0.02	0.890	0.18	0.674	1.09	0.302	0.69	0.409	0.44	0.512	0.25	0.619
Juvenile (4 months)												
Number leaves ($NoLVS_4$)	0.08	0.772	0.27	0.607	0.00	0.963	1.96	0.161	0.49	0.485	3.19	0.074
Rosette height ($RosHT_4$) (mm)	0.74	0.389	2.53	0.112	0.38	0.536	1.20	0.273	0.70	0.402	0.00	0.953
Adult (8 months)												
Number leaves ($NoLVS_8$)	0.67	0.415	1.32	0.251	0.21	0.644	0.42	0.515	0.58	0.447	3.33	0.068
Plant height (HT_8) (mm)	0.32	0.572	1.49	0.222	2.35	0.125	0.14	0.710	0.17	0.679	0.03	0.861
Reproductive adult (12 months)												
Number of flowers & buds ($NoF\&B_{12}$)	0.19	0.664	0.00	0.947	3.37	0.066	1.22	0.270	1.21	0.272	0.12	0.734
Total biomass (TB_{12}) (g)	0.07	0.794	1.79	0.181	2.85	0.091	0.19	0.666	0.14	0.704	3.81	0.052
Cumulative fitness index (CFI_{12})	0.01	0.938	**4.87**	**0.033**	1.34	0.255	0.02	0.890	1.91	0.177	2.11	0.153

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