Fitness consequences of hybridization between ecotypes of *Avena barbata*

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Hybridization is an important factor in the evolution of plants; however, many of the studies which have examined hybrid fitness have been concerned with the study of early generation hybrids. Three studies which examined the fitness consequences of hybridization between two ecotypes of *Avena barbata* were carried out. The first experiment, which examined the short and long term consequences of hybridization, determined that hybrid vigour which counteracts hybrid breakdown occurs in the F2 generation. Also, although the F6 generation mean is lower than the mid-parent mean, there are individual genotypes within the F6 generation which are capable of outperforming the parental ecotypes. The second experiment examined the fitness of the F6 generation in novel environments, demonstrating that certain genotypes do better under certain environmental conditions and that there are genotypes capable of outperforming the parental ecotypes in all the novel environments tested. The third experiment examined the fitness of early and late generation hybrids in the parental environments, confirming the results of the first experiment which demonstrated the presence of hybrid vigour counteracted by hybrid breakdown in the F2 generation. However, there do not appear to be genotype by environment interactions for fitness. While there are a small number of hybrid genotypes which are capable of outperforming the parental ecotypes in the parental environments, the mesic ecotype outperformed the xeric ecotype in the xeric environment, calling into question whether these ecotypes are locally adapted to their specific environments. Overall, these experiments demonstrated that a single hybridization event can result in a number of outcomes including hybrid vigour, hybrid breakdown, transgressive segregation and genotype by environment interactions.

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Chapter 1

Introduction

Historically there have been two contrasting views on the importance of hybridization. One view is that hybridization is an important factor driving evolution and speciation (Arnold 1997). This view holds that it is possible not only for hybrids to be more fit than either of the parents but also that stable hybrid zones can occur (Moore 1977). In order for the hybrids to have a fitness greater than the parental phenotypes it is necessary for the hybrid to be better adapted to, or for the parents to be less adapted to certain environmental conditions (Moore 1977). This view has been supported by studies which demonstrate that certain hybrids are in fact more fit under certain environmental conditions (Emms and Arnold 1997) and that hybridization can lead to speciation (Rieseberg *et al.* 1990; Rieseberg 1991; Schwarzbach *et al.* 2001). A review of the literature by Arnold and Hodges (1995) found that not only was it possible for hybrids to be more fit than both parents, but that hybrids were more fit than one parent in over half the cases. That hybrids which are adapted to different environmental conditions than their parents can ultimately lead to speciation has been shown to occur in *Helianthus anomalus*. *H. anomalus* is the putative hybrid offspring of *H. annuus* and *H. petiolaris* and has many characteristics which might have resulted in this hybrid being able to adapt to an environment different from that of the parental environments (Schwarzbach *et al.* 2001).

The opposite view is that hybrids are generally less fit than their parents and relatively unimportant from an evolutionary stand point (Wagner 1970). While hybrids

might form, and may be common, adherents of this view hold that they are for the most part sterile (Wagner 1970; Mayr 1992). If a hybrid does survive, it will occupy a habitat which is different from the parental habitat and will disappear once the parental species has re-established itself in its usual environment (Wagner 1970). Therefore, while hybrids do exist, it is the "normal" parental species which are assumed to contribute to evolution (Wagner 1970; Mayr 1992).

However, it is perhaps more accurate to say hybridization has multiple outcomes with everything from the production of F1 hybrids, which are inviable, to frequent hybridization resulting in viable offspring (Avise 1994; Arnold 1997). The overall outcome of the hybridization event will depend on whether selection is exogenous (interactions between the hybrid genotype and the environment) or endogenous (interactions between parental genomes). If the two parental phenotypes have co-adapted gene complexes which are independent of the environment, then the hybrids will likely have a lower fitness than the parents as a result of the disruption of these gene complexes (Moore 1977). However, if parental phenotypes have co-adapted gene complexes which are associated with a specific environment, then the resulting hybrids may survive if they are not competing with the parents in the parental environment (Moore 1977).

In spite of this debate, numerous studies have documented the occurrence of hybridization both in nature and between crops and wild relatives. For example hybridization between two subspecies of the grasshopper *Caledia captiva* has been well documented (Shaw *et al.* 1983; Shaw *et al.* 1986; Marchant *et al.* 1988). The two subspecies Torresian and Moreton mate and form a hybrid zone where the two ranges meet (Marchant *et al.* 1988). However, after the F1 generation, hybrid breakdown seems

common. The F2 generation is almost completely inviable and backcrossing between the parents and F1 results in viability being reduced by 50% (Marchant *et al.* 1988). Despite this it appears that some introgression of molecular markers has occurred from the Moreton population into the Torresian population (Marchant *et al.* 1988). This introgression may have occurred as a result of the movement of the hybrid zone and selection against the disruption of co-adapted cis-acting gene complexes (Marchant *et al.* 1988). *Hyla cinerea* and *H. gratiosa* are known to hybridize despite the differences in habitat between the two (Lamb and Avise 1986). Most of the F1 hybrids have *H. gratiosa* as the maternal parent, possibly due to the position of the males during mating season (Lamb and Avise 1986). It appears that the hybrid offspring are stable in terms of their morphological development. However, as this work was carried out on F1 hybrids and backcrosses, whether similar stability would be found in later generation hybrids can not be inferred (Lamb et al. 1990). *Bombina bombina* and *B. variegata* are two species which hybridize and have been studied extensively. The outcome of hybridization between these two species seems to depend both on exogenous and endogenous selection (Arnold 1997) with lower fitness of the hybrids and contrasting environments on either side of the hybrid zone resulting in introgression occurring over a very narrow zone (Szymura 1996). Fitness of the hybrids between these two species varies with some hybrid genotypes showing no reduction in viability while others resulted in zero viability (Kruuk *et al.* 1999).

Hybridization in plants is also well documented. *Helianthus anomalus*, *H. deserticola* and *H. paradoxus* are all thought to be stabilized hybrid derivatives of *H. annuus* and *H. petiolaris* with both molecular data and the geographical distribution of

the three hybrid species supporting this hypothesis (Rieseberg 1991). The distribution of these three species is not intermediate to that of the parental forms, but rather the habitats they occupy are extreme to those of the parental species (Rieseberg 1991). *H. anomalus* and *H. deserticola* are both adapted to xeric conditions whereas *H. paradoxus* occurs in brackish, saline and marine environments (Rieseberg 1991). Hybrid speciation of *H. anomalus* appears to have occurred in less than 60 generations, indicating that under favourable conditions speciation can occur quite quickly (Ungerer *et al.* 1998). Hybridization has been reported to occur between *Iris* spp. as well. *Iris nelsonii* is the putative stabilized hybrid derivative of *I. fulva* and *I. hexagona*, which appears to have occurred as a result of crosses involving *I. fulva* as the maternal parent and *I. hexagona* as the paternal parent (Arnold *et al.* 1991). Also, hybridization occurs between *I. fulva*, *I. hexagona* and *I. brevicaulis* with introgression between *I. fulva* and *I. brevicaulis* being bi-directional (Arnold et al. 1992). Overall, introgression between these three species seems to be pollen-mediated as opposed to through seed dispersal of one species into the habitat of another species (Arnold *et al.* 1992). A further study has found that while F1 production of hybrids between *I. fulva* and *I. hexagona* is a rare event, if an F1 can become established in a population it will backcross and produce F2 offspring at a much greater rate than the F1s themselves are produced (Hodges *et al.* 1996). This indicates that although there may be difficulty in establishing the initial hybrid, once there has been the formation of an F1 this can lead to the rapid formation of a hybrid zone (Hodges *et al.* 1996).

Also, hybridization has been well documented in crop species. Canola (*Brassica napus*, 2n=38) has been found to successfully cross with several wild species, including

B. rapa, *B. juncea*, *Hirschfeldia incana*, *Sinapis arvensis* and *Raphanus raphanistrum*, after hand pollination (Rieger *et al.* 1999). The cultivated radish *R. sativus* has been shown to hybridize naturally with both feral *R. sativus* as well as *R. raphanistrum* (Klinger *et al.*1992; Klinger and Ellstrand 1994; Snow *et al.* 2001). Sugar beets (*Beta vulgaris* ssp. *vulgaris*) hybridize naturally with adjacent populations of wild sea beet (*B. vulgaris* ssp. *maritima*) as well as with a weedy form of *B. vulgaris* ssp. *vulgaris* (Bartsch *et al.* 1999; Desplanque *et al.* 1999; Desplanque *et al.* 2002; Lavigne *et al.* 2002; Viard *et al.* 2002). These are but a few of the studies which have demonstrated that hybridization does occur both in nature and under crop conditions. These studies have shown that hybridization and the subsequent introgression of genes from one species to another is a frequent event in the evolution of species (Rieseberg and Ellstrand 1993; Nason and Ellstrand 1993; Rieseberg *et al.* 2000). However, few of these studies have looked at the long term fitness consequences or the underlying genetic basis of this fitness.

The outcome of any hybridization event will depend in part on which of the two seemingly opposite fitness consequences of hybridization occur. Heterosis (hybrid vigour) is the superiority of the hybrid offspring over the mid-parental value for that trait (Stuber *et al.* 1992; Rieseberg *et al.* 2000), whereas hybrid breakdown is the sterility and weakness in the F1 and later generation hybrid offspring (Li *et al.* 1997). Heterosis and hybrid breakdown are not mutually exclusive. While a cross may exhibit heterosis in the early generation hybrid offspring, this may give way to hybrid breakdown in later generation hybrids as recombination disrupts co-adapted gene complexes or results in the creation of recessive deleterious homozygotes (Rieseberg *et al.* 2000). Whether heterosis

or hybrid breakdown occurs will depend on the relative amounts of additive, dominance and epistatic gene effects.

Although hybridization is often thought of as a cross between two different species, which results in either highly sterile F1 individual or in individuals that can only occupy habitats intermediate to that of the parents (Mayr 1992), an alternative viewpoint defines hybridization as any mating in nature between individuals from groups that are distinguishable based on one or more heritable characters (Arnold 1997). This definition includes matings between conspecific individuals but not individuals from the same gene pool and does not require acceptance of any particular species concept (Arnold 1997).

Genetic factors important to hybrid fitness

The genetic basis of fitness, as well as other phenotypic traits, of the hybrid offspring can be understood in the context of line crosses. Expectations for each generation of a line cross and thus each generation of hybrid offspring have been derived by several authors (Mather and Jinks 1982; Lynch and Walsh 1998). All of these expectations assume that all the "plus" alleles, meaning those alleles that increase the trait value, are fixed in one parent while the other parent is fixed for all the "minus" alleles, meaning those alleles that decrease the trait value. The use of these expectations for each generation can help to determine the relative contributions of additive, dominance and epistatic gene effects to the fitness of the hybrid offspring (Lynch and Walsh 1998).

The phenotype consists of the sum of additive and dominance gene effects at individual loci plus the epistatic interactions between loci (Mather and Jinks 1982). The means of each generation given in Table 1.1 are expressed as fractions of the additive

Table 1.1: Proportion of additive, dominance and epistasis contributing to trait mean each generation. Modified from Mather and Jinks (1982).

$P1 = m + A + AxA$
$P2 = m - A + AxA$
$MP = m + AxA$
$F1 = m + D + DxD$
$F2 = m + 1/2D + 1/4DxD$
$F_{n+1} = m + (1/2)^{n}D + (1/2)^{2n}DxD$
$F6 = m + 1/32D + 1/1024DxD$ = approximately m
$BC1_{p1} = m + 1/2A + 1/2D + 1/4AxA + 1/4AxD + 1/4DxD$
$BC1_{p2} = m - 1/2A + 1/2D + 1/4AxA - 1/4AxD + 1/4DxD$
$BC2_{p1} = m + 3/4A + 1/4D + 9/16AxA + 3/16AxD + 1/16DxD$
$BC2_{p2} = m - 3/4A + 1/4D + 9/16AxA - 3/16AxD + 1/16DxD$

m = average of recombinant inbred line (RIL)

A = sum of all additive gene effects

AxA = sum of all pairs of additive interactive loci

D = sum of all dominant gene effects

DxD = sum of all pairs of dominant interacting loci

AxD = sum of all pairs of additive and dominant interacting loci

$BC1_{p1}$ = F1 x P1

$BC1_{p2}$ = F1 x P2

$BC2_{p1}$ = $BC1_{p1}$ x P1

$BC2_{p2}$ = $BC1_{p2}$ x P2

MP = mid-parent

and dominance gene effects plus the interaction terms. The contribution of each of these terms is derived from relative frequencies of the genotypes in each generation and therefore will change depending on the generation (Table 1.2) (Mather and Jinks 1982).

Additive Gene Effects

In the absence of either dominance or epistasis the phenotype will be the result of the sum of the additive gene effects across loci (Wade 2001). The expectations given in Table 1.1 assume the parental lines are fixed for opposite alleles at all loci; therefore, the parental lines do not exhibit any dominance effects. The sign associated with the additive gene effects indicates whether the alleles increase or decrease the character in question (Mather and Jinks 1982). If the parents are fixed for opposite alleles such that the variation in a trait is purely additive, each generation of hybrid offspring (not including the backcross generation) of these two true breeding lines should have a phenotype midway between that of the parents (Mather and Jinks 1982). Additive gene effects are not included in the expectations for the F2 and later generation means because if the parents are fixed for opposite alleles then $p=q=0.5$ and any additive gene effects will cancel out. However, as the effects are summed across all pairs of loci, even if the alleles are dispersed in the parental generations, meaning that each parent carries an increasing allele for one gene and the decreasing allele for another gene, the expectations for the F1 and subsequent selfing generations will remain the same as the expectations given in Table 1.1 (Mather and Jinks 1982).

Table 1.2: Relative frequencies of genotypes across generations and contributions of additive, dominance and epistatic gene effects.

	Genetic Effects			Frequencies							
Genotype	Additive	Dominant	Epistatic	$Parent_1$	$Parent_2$	F1	F2	$BC1_{p1}$	$BC1_{p2}$	$BC2_{p1}$	$BC2_{p2}$
AABB	$A_A + A_B$		A x A	1	0	0	1/16	1/4	0	9/16	0
AABb	A_A	D_B	A x D	0	0	0	1/8	1/4	0	3/16	0
AAbb	A_A - Ab		A x - A	0	0	0	1/16	0	0	0	0
AaBB	A_B	D_A	A x D	0	0	0	1/8	1/4	0	3/16	0
AaBb		$D_A + D_B$	D x D	0	0	1	1/4	1/4	1/4	1/16	1/16
Aabb	$-A_b$	D_A	-A x D	0	0	0	1/8	0	1/4	0	3/16
aaBB	$-A_a + A_B$		-A x A	0	0	0	1/16	0	0	0	0
aaBb	$-A_a$	D_B	-A x D	0	0	0	1/8	0	1/4	0	3/16
aabb	$-A_a + -A_b$		-A x -A	0	1	0	1/16	0	1/4	0	9/16

Dominant Gene Effects

Interactions between alleles at the same locus are referred to as dominant gene effects and result in the phenotype of the heterozygotes being different from the mid-parent phenotype (Mather and Jinks 1982). As the F1 resulting from a cross of two homozygous parents are entirely heterozygous, the F1 performance relative to the parents is going to be made up of dominance effects as well as any dominance x dominance epistasis that may be present (Table 1.1). If the parental populations are homozygous for deleterious recessive alleles at different loci, then combining the two genomes may result in dominant gene effects masking the deleterious recessive alleles. This will result in F1 hybrids that exhibit heterosis (Whitlock *et al.* 2000). Heterosis is more likely to occur if the dominance effects are in a positive direction at all loci, which means the dominance effects at all loci result in the masking of deleterious alleles and an increase in the trait in question. If the dominance effects are not unidirectional at all loci, then negative dominance effects at some loci may cancel out any positive effects of the masking of deleterious alleles at other loci (Mather and Jinks 1982). The F2 generation exhibits half the dominant gene effects of the previous generation and as heterozygosity decreases by one half every generation the F_{n+1} will only exhibit $(1/2)^n$ dominant effect. Therefore, if dominance is contributing to heterosis in the early generation hybrids then, with the loss of heterozygosity, the later generation hybrids will lose this fitness advantage.

Epistatic Gene Effects

Epistasis occurs when there are interactions between genotypes at different loci. With epistasis the effect of a genotype at one locus will depend on what genotype is present at the other locus (Mather and Jinks 1982). Hybrid breakdown may occur through

unfavourable interactions between the two parental genomes in the F1 generation (Burke and Arnold 2001) or through the break-up of co-adapted gene complexes which were present in the parents (Waser and Price 1994). Although co-adapted gene complexes are assumed to be favourable epistatic combinations, not all epistatic interactions result in an increase in the trait value. Therefore, while some additive x additive epistatic interactions will result in an increase in the trait value, others may not. As the parents are assumed to be fixed for alleles of opposite effects, the additive gene effects will cancel each other out in the later generation hybrids and therefore do not contribute to the generation means (Table 1.2). Therefore, as the coefficients of the epistatic interaction terms are the products of the coefficients of the non-interaction terms (Mather and Jinks 1982) the additive x additive epistatic effects are not included in the generation means for the F2 and later generation hybrids. Although these terms are not reflected in the overall mean of the generation, both the additive gene effects and the epistatic interactions may still contribute to the variances for each generation. It is possible that there may be some hybrid individuals in which the production of new beneficial epistatic gene combinations may result an increase in fitness (Burke and Arnold 2001). However, in the absence of strong selection, later generation hybrids may lose this fitness advantage as recombination breaks up the favourable gene combinations (Burke and Arnold 2001).

Local Adaptation and Transgressive Segregation

If additive gene effects are contributing to local adaptation then hybridization between two individuals which are not adapted to the same environment may result in an F1 fitness lower than the local parent (Waser and Price 1994). This, like any additive gene effect, is due to the fixation of alleles of opposite effect in the parents. Therefore,

the fitness of the mid-parent will equal the fitness of the F1, but will be less than the fitness of either parent in the parental habitat. If the alleles which are present in one parent are beneficial in one environment but are not beneficial in another environment, then the immigration of these non-native alleles will erode adaptation to the local environment.

Nevertheless, although the mean of the F2 generation may be no different from the mean of the mid-parent, the segregation of additive genetic variation in the F2 and later generations sometimes results in extreme phenotypes relative to the parents (transgressive segregation) in some individuals (Rieseberg *et al.* 1999). These transgressive phenotypes may arise through accumulation of complementary alleles at several loci in some individuals (Tanksley 1993; Rieseberg *et al.* 1999). Complementary gene action occurs when alleles of opposing effects are fixed within parental lines (deVicente and Tanksley 1993; Rieseberg *et al.* 1999). For example, for a particular trait one parent may be fixed for three "plus" alleles and two "minus" alleles giving an overall phenotypic value of plus one, while the other parent may be fixed for three "minus" alleles and two "plus" alleles giving an overall phenotypic value of minus one. The resulting F2 and later generation hybrid offspring may contain individuals who have all five of the "plus" alleles or all five of the "minus" alleles resulting in some individuals which have extreme phenotypes relative to either parent. Although evidence usually points to complementary gene action as an explanation for transgressive segregation, epistatic interactions or overdominance could also contribute to the phenotypes of these extreme individuals (Tanksley 1993; Rieseberg *et al.* 1999). Epistatic interactions may actually be making a substantial contribution to transgressive segregation. However, the

QTL mapping methods which are used in these studies have a low power for detecting epistasis (Burke and Arnold 2001). The overall outcome of the presence of transgressive segregation may be that these extreme phenotypes result in either adaptations to a novel environment or in novel traits in the hybrid offspring (Rieseberg *et al.* 1999). If the hybrid offspring exhibit novel adaptations, they may be able to colonize a non-parental environment leading to the establishment of the hybrid lineage in an area previously un-colonized by this species.

Backcross Generations

When the F1 is backcrossed to one of the original parents to create the first backcross generation, half of the additive effect of the parent used in the cross is expressed. This occurs through crossing the heterozygous F1 with a homozygous parent resulting in the re-creation of some of the homozygous loci which were previously present. However, this cross also results in the loss of heterozygosity relative to the F1 so only half the dominance effect of the F1 generation is expressed (Table 1.1,1.2). In the second backcross generation the offspring may start to exhibit phenotypes more like the original parent. This is due to the increased homozygosity of this generation and therefore the return of the additive effects and additive x additive epistatic interactions which were lost in the F1 generation. The dominance x additive epistatic interactions may contribute to either an increase or decrease in fitness of the offspring depending on whether the epistatic interactions are positive or negative. Only a quarter of the dominance effects which were present in the F1 will be seen in the BC2 generation, as crossing to the original parent decreases the proportion of heterozygotes. Therefore, if dominance effects are contributing to heterosis in the F1 generation there will be a decrease in the fitness of

the backcross generations relative to the F1 with each successive generation of backcrossing.

Summary of literature documenting outcomes following hybridization

The relative contributions of additive, dominant and epistatic gene effects are going to determine the fitness of any hybrid offspring. The occurrence of heterosis or hybrid breakdown is a function of a number of factors such as whether there are interactions between parental genomes, whether additive gene effects confer adaptation to the local environment and whether there are recessive deleterious alleles in the parental populations.

All of these outcomes have been documented as occurring following hybridization events either between crop plants and their wild relatives or between two non-cultivated species. Heterosis has been found to occur upon hybridization between weedy *Raphanus sativus* and crop *R. sativus*. The resulting F1 have higher fruit and seed production than their wild counterparts in a non-competitive situation (Klinger and Ellstrand 1994). The F1 and F2 hybrids of *I. fulva* and *I. hexagona* were found to survive and reproduce just as well as, and sometimes better than the parents in the parental environments (Emms and Arnold 1997). As well, heterosis has been reported in a number of other plant species including *Arnica montana*, *Gentiana pneumonanthe*, *Agrostemma githa* and *Silene alba*, as reviewed in Hufford and Mazer (2003). Estimations of epistatic effects found that heterosis for weight in eight week old mice was caused mainly by beneficial recombinant gene combinations (Mohamed *et al.* 2001).

Heterosis does not always occur in the F1 generation; it is also possible that hybrid breakdown will occur in these early generation hybrids. F1 hybrids formed by

hand pollination between the wild radish *R. raphanistrum*, and *R. sativus* were found to flower later, have lower pollen viability, and have only half as many seeds per fruit as their wild progenitor when grown both in potted conditions and under field conditions (Snow *et al.* 2001). *Cucurbita pepo* occurs both as a wild and a cultivated species and although seedling survival of the crop-wild F1 hybrid offspring is no different from that of the wild parent, male and female flower number as well as seed number were lower than in the wild parent (Spencer and Snow 2001). As well, unfavourable interactions between *H. annuus* and *H. petiolaris* appear to negatively affect the fertility of F1 hybrids between these two species (Rieseberg *et al.* 1996).

However, it is possible for both heterosis and hybrid breakdown to occur in the same cross. Hybrid breakdown has often been found following heterosis in the early generation hybrids. The F1 offspring resulting from a cross between *Brassica napus* and *B. rapa* had higher seed set than either parent (Hauser *et al.* 1998a; Pertl *et al.* 2002). However, the F2 and backcross generations had a lower fitness with less viable pollen and fewer seeds than the parents (Hauser *et al.* 1998b). Hybridization between native and immigrant song sparrows on Mandatre Island resulted in heterosis in the F1 hybrids, but the performance of the F2 hybrid was less than the average of the F1 and mid-parent value indicating the break-up of co-adapted gene complexes (Marr *et al.* 2002). F1 hybrids of *Chamaecrista fasciculata* were found to exhibit heterosis and outperformed the parents across a range of sites, although the amount of heterosis exhibited depended on the site in question (Fenster and Galloway 2000a,b). The disruption of epistatic interactions lead to hybrid breakdown in later generations however, with the F3 hybrids

exhibiting a fitness which was less than the average of the F1 generation and mid-parent value (Fenster and Galloway 2000b).

The fitness of the hybrid offspring is not completely independent of the environment but will depend on the presence of genotype x environment interactions. Local adaptation is a wide-spread phenomenon seen in both plants and animals and studies which document this phenomenon have been carried out for over a century. Reciprocal transplant experiments have demonstrated that numerous plant species are locally adapted to their specific environments. For example *Potentilla gladulosa* and *Achillea millefolium* are differentiated into climatic races which perform best in their native environment (Clausen *et al.* 1941). As well, *Agrostis tenuis*, *Trifolium repens*, *Anthoxanthum odoratum*, *Ranunculus repens*, *Festuca ovina* are all locally adapted to their native conditions (Reviewed in Bradshaw 1984). Turesson (1923) studied local adaptation by performing reciprocal transplants of more than 1200 individuals of *Hieracium umbellatum*. Local adaptation has also been found in *Ceanothus* sp. (Nobs 1963), *Ranunculus lappaceus* (Briggs 1962), subspecies of *Dactylorchis incarnate* (Heslop-Harrison 1956) as well as within *Achillea borealis* subspecies *Californica* (Kruckeberg 1954) to name a few. If the parental populations are genetically distinct from one another and locally adapted, hybridization may result in the loss of adaptation to the parental environment and thus the F1 hybrid may have reduced fitness. *Artemisia tridentata* ssp. *tridentata* and *A. tridentata* ssp. *vaseyana* hybridize and form a narrow hybrid zone where the two subspecies meet (Wang *et al.* 1997). Seeds from hybrids within this zone were collected and used in a reciprocal transplant experiment along with the two parental subspecies. The hybrids were found to outperform the parents within the

hybrid zone, which is distinctly different from either of the parental sites in terms of soil composition, and the parent subspecies outperformed hybrids and the other parental subspecies in each of their natural environments (Wang *et al.* 1997). Reciprocal transplants of the two subspecies *Gilia capitata* ssp. *capitata* and *G. capitata* ssp. *chamissonis* revealed that although selection against the immigrant was stronger in one environment than in the other, there was local adaptation for emergence, flowering and inflorescence number, with the native subspecies performing better than the immigrant seed in both environments (Nagy and Rice 1997). When F2 hybrids were grown in each of the parental environments, selection was found to favour the native phenotype with one exception, selection for leaf shape at one site was towards the immigrant morphology (Nagy 1997). In *Iris brevicaulis* and *I. fulva*, the *I. brevicaulis*-like hybrids and *I. fulva*-like hybrids are more likely to be found in habitat similar to the habitat of the closest parental species (Johnston *et al.* 2001a). Also, species-specific molecular markers which are associated with the environmental conditions of the parents were found to be correlated with the environment in the hybrid classes suggesting environment-dependent selection in the hybrid (Johnston *et al.* 2001a). F1 hybrids of the limnetic and benthic forms of the stickleback have been found to perform well in laboratory conditions although reciprocal transplants of the parents and F1 hybrids in their natural environments found that the parents grew better in their individual environments and growth of the F1 hybrids was below the mid-parent value (Schluter 1995; Hatfield and Schluter 1999). This led to the conclusion that selection is acting against intermediate phenotypes of sticklebacks in the wild (Hatfield and Schluter 1999).

Alternatively, hybridization may result in the production of novel gene combinations that do just as well, or sometimes better than the parental populations. This in turn may lead to range expansion or species formation (Potts and Reid 1988; Nason and Ellstrand 1993). Molecular evidence indicates that *Helianthus anomalus*, *H. deserticola* and *H. paradoxus* are stabilized hybrid derivatives of *H. annuus* and *H. petiolaris* ssp. *fallax* (Rieseberg 1991). While a segment of the habitat of each of these three hybrid species overlaps with the habitats of both the parental species, the three hybrid species prefer habitats which are extreme relative to that of the parental species (Rieseberg 1991). The molecular data together with the geographical data of these three species indicate that hybridization can lead both to species formation and range expansion. Further evidence that hybridization can lead to both species formation and range expansion can be found in the Louisiana irises. *Iris nelsonii*, the stabilized hybrid derivative of *I. fulva* and *I. hexagona*, arose with *I. fulva* as the maternal parent and occupies a unique habitat relative to the parental species (Arnold *et al.* 1990; Arnold *et al.* 1991).

Research Objectives

The purpose of my research was to examine the evolutionary fate of hybrids formed between two putative locally adapted forms of *Avena barbata*. Three questions were addressed. The first question looked at the short and long term consequences of hybridization in *A. barbata*; the second question addressed whether the novel genotypes formed by recombination in the cross have novel characteristics and if the success of these genotypes depended on adaptation to a particular environment; the third question examined the performance of early and late generation hybrids in the parental

environment. These experiments together address many of the main issues of hybridization, including the long term fate of the hybrids, whether dominance and epistasis play a role in hybrid fitness, the importance of examining the fitness of individual genotypes as opposed to simply looking at generation means, and whether hybrid breakdown and hybrid vigour are mutually exclusive.

Study Organism

A. barbata is a diploidized tetraploid (2n=4x= 28) annual grass with a high level (greater than 95%) of self-fertilization (Allard 1999). It was first introduced to California from the Mediterranean approximately 200 years ago (Clegg and Allard 1972; Hamrick and Allard 1972; Allard 1999). *A. barbata* is believed to have originated as a result of polyploidization in the *A. hirtula-A. weistii* species complex (Hutchinson *et al.* 1983; Allard *et al.* 1993). *A. barbata* occurs in habitats ranging from relatively fertile, mesic conditions to infertile xeric conditions. Two ecotypes were used in this study; one was considered a mesic ecotype, the other a xeric ecotype. Ecotype is used to refer to a genotype within a species which is adapted to certain environmental conditions (Smith 1986; Hufford and Mazer 2003). These two ecotypes can be distinguished based on five allozyme loci (leucine amino peptidase-1, esterase-1, acid phosphatase-1, 6-phosphogluconate dehydrogenase and peroxidase-1) (Hutchinson *et al.* 1983). The two ecotypes can also be distinguished based on morphological characteristics, with most xeric populations exhibiting glabrous leaf sheaths and dark lemmas, and the mesic populations exhibiting hairy leaf sheaths and light lemmas (Allard 1996). The pattern of association between these allozyme loci and the environment has been found to be non-random with plants that occur in mesic regions tending to be fixed for one set of alleles

while plants from the xeric regions tend to be fixed for the opposite set of alleles (Clegg and Allard 1972). This pattern of association has been found throughout California between regions as well as on a scale of only a few feet within these regions (Allard *et al.* 1972). Patches of polymorphism for the xeric and mesic genotypes can be found where the two habitats meet, with the two genotypes being correlated with the level of moisture (Garcia *et al.* 1989). The frequency of each genotype within a given area may shift from year to year depending on the environmental conditions (Perez de la Vega *et al.* 1991; Allard *et al.* 1993). There may be a shift from predominately xeric genotype to predominately mesic genotype if a year of very dry weather is followed by a year of plentiful rainfall (Allard *et al.* 1993).

Similar patterns of association between genotype and environment are found in the Mediterranean populations (Garcia *et al.* 1989). However, while the California population and the Mediterranean populations are similar in both allelic composition and frequency, the multi-locus genotypes which are found in the California population are quite different from those found in the Mediterranean population (Garcia *et al.* 1989; Perez de la Vega *et al.* 1991). As the ancestral Spanish populations are highly polymorphic but do not contain the genotypes found in California, the genotypes exhibited by the Californian populations appear to have arisen through recombination subsequent to the arrival of *A. barbata* in California (Garcia *et al.* 1989; Perez de la Vega *et al.*1991; Allard 1999). These non-random associations among alleles has led to the hypothesis that selection has not only resulted in the pattern of association between the multi-locus genotypes and the environment (Clegg and Allard 1972; Hamrick and Holden 1979; Garcia *et al.* 1989; Perez de la Vega *et al.* 1991) but also that epistatic

interaction among alleles at these loci may be important in adapting to current habitats and maintaining the genetic variability of this species (Clegg and Allard 1972). However, it is also possible that while selection results in an association between the five allozyme loci and the environment, it is possible that selection is not acting directly on these five allozyme loci but rather on a locus which is linked to these allozyme loci (Hamrick and Holden 1979; Hedrick and Holden 1979).

These previous studies which examined *A. barbata* and the putative co-adapted gene complexes were carried out by surveying natural populations and did not examine hybrids formed between the two ecotypes. The current study will examine both the mesic and xeric ecotypes as well as hybrid offspring which have been propagated to the F6 generation. Producing hybrids between these two ecotypes will disrupt any co-adapted gene complexes which do exist. Therefore, the use of the F6 generation should allow for a more clear understanding of whether co-adapted gene complexes do exist and whether they are involved in the fitness of the ecotypes. As *A. barbata* is an annual plant, the number of seeds produced by each plant will give a measure of the lifetime reproductive success of each plant.

The parental (mesic and xeric) seeds used to create the crosses were made available to us by Dr. Pedro Garcia. These seeds were collected in California in the late 1980s and were known to have the classic mesic and xeric genotypes. The experimental lines were created by crossing both a homozygous mesic as the pollen donor and a homozygous xeric as the pollen recipient and a homozygous mesic as the pollen recipient with a homozygous xeric as the pollen donor. Crosses were carried out by emasculation of the floret, followed within 24 hours by hand pollination. Six heterozygous F1 hybrids

(five from a mesic pollen donor X xeric pollen recipient cross, one from a xeric pollen donor X mesic pollen recipient cross) were allowed to self-fertilize to produce the F2 generation. Two hundred F2 seeds from one of these families (F2-A) were allowed to self fertilize to produce F3 hybrids. One F3 seed from each F2 plant was grown to produce the F4 generation. This procedure of single seed descent was carried out to the F6 generation (Figure 1.1). For the purposes of this research the term parental ecotype will be used when referring to the mesic and xeric ecotypes. Family is used to refer to all seeds from a single parent. Thus, all the F2 seeds which were produced from a single F1 are considered a family, hence there are six F2 families (F2A-F). All F3 seeds which resulted from a single F2 seed are referred to as a family resulting in 200 F3 families. This definition of family carries through to the F6 generation, meaning there are 200 F6

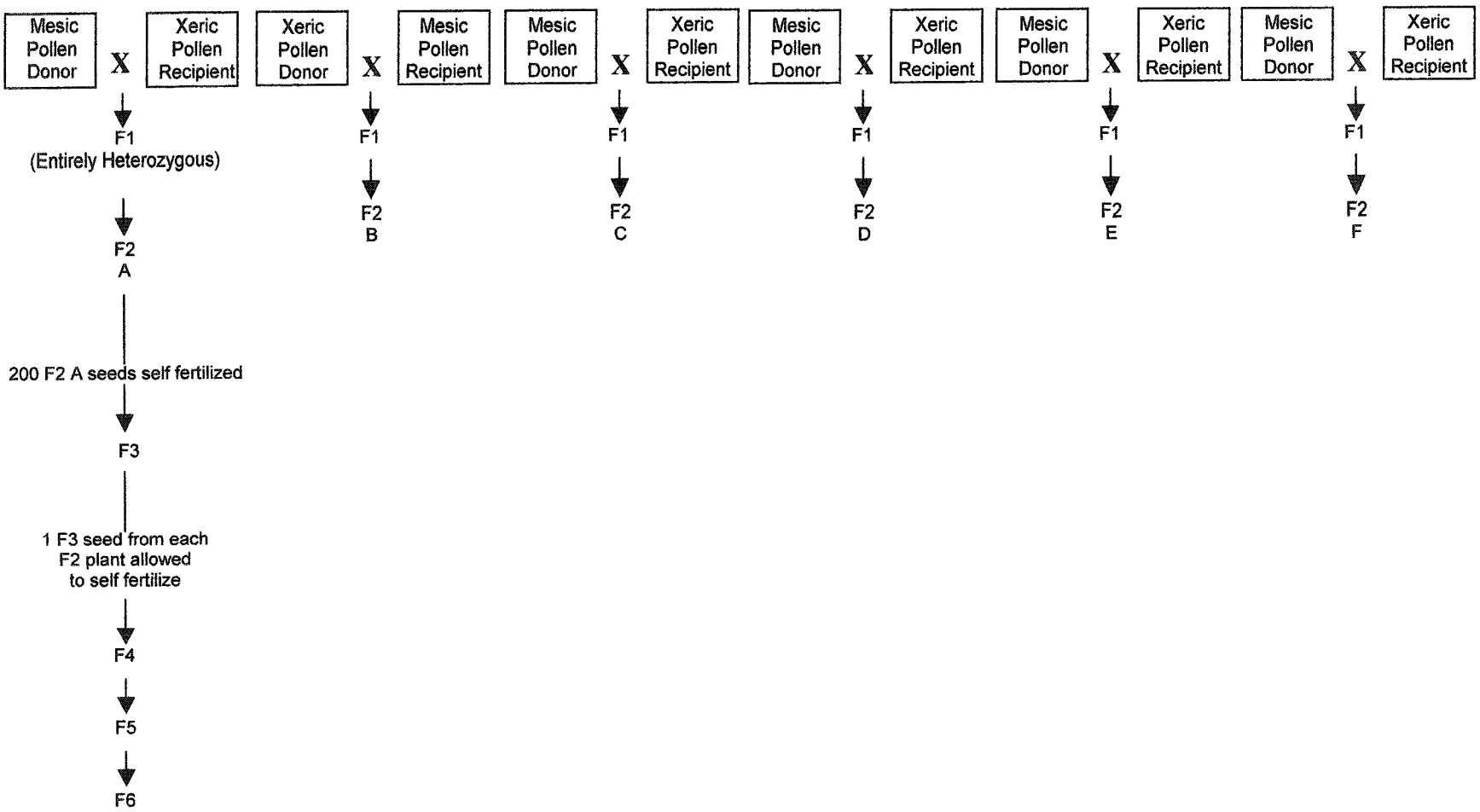

Figure 1.1: Crossing design for the production of the experimental lines between two ecotypes of *A. barbata*.

families descended from the original 200 F2 seeds. Twenty-five of these 200 families were randomly chosen to be used in my research.

Chapter 2

Short and long term effects of hybridization in *Avena barbata*

Introduction

Natural hybridization is frequently regarded as a process which results in non-viable individuals or individuals which are less fit than the parents (Arnold *et al.* 1999). However, hybridization has several outcomes, which can result from one single cross and are not mutually exclusive (Chapter 1). While some hybridization events do cause the break up of co-adapted gene complexes, which in turn results in hybrid breakdown, producing individuals with an average lower fitness than their parents, other events will result in individuals that are on average just as fit, if not more fit, than the parents. This hybrid vigour may arise through dominance, overdominance or epistasis (Stuber *et al.* 1992; Mitchell-Olds 1995). However, many crosses may result in some combination of the two in different generations following the original hybridization event.

While the fitness of the hybrid offspring may be on average either higher or lower than the parental fitness, it is possible that individual genotypes within the recombinant hybrid offspring may do much better or worse than the most fit or least fit parent. This outcome, which may affect only some of the hybrid offspring is referred to as transgressive segregation. Transgressive segregation is the presence of extreme phenotypes in either a negative or positive direction relative to the parental phenotypes (Rieseberg *et al.* 1999, 2000; Tanksley 1993). As with hybrid breakdown, the production of these extreme phenotypes is the result of recombination, and therefore both hybrid breakdown and transgressive segregation can result from the same cross and occur in the

same generation. The production of these extreme phenotypes may contribute to novel adaptations which may allow the hybrid offspring to develop niche differentiation from their parents, resulting in their establishment as either stabilized introgressants or hybrid species (Rieseberg *et al.* 1999, 2000). The niche differentiation will allow for less competition from the parents as well as possible reproductive isolation (Rieseberg *et al.* 1999). The production of transgressive phenotypes is most often attributed to complementary gene action which results when the parental lines are fixed for alleles with opposing effects which increase the trait at some loci and decrease it at others (Rieseberg *et al.* 1999; deVicente and Tanksley 1993; Tanksley 1993). The resulting hybrid offspring may have genotypes that combine positive (or negative) alleles from the two parents, producing a phenotype which is extreme relative to the parents. Even though epistatic interactions have been shown to occasionally play a role in transgressive segregation (Rieseberg *et al.* 2003) it has not commonly been found to be a major cause of the phenomenon (Rieseberg *et al.* 1999). Whether this is because epistasis does not contribute to transgressive segregation very often or because it is difficult to detect is not known (Rieseberg *et al.* 1999).

If a certain set of alleles is found to work well together and are therefore selected for, this can lead to the production of co-adapted gene complexes (Fenster *et al.* 1997). Co-adapted gene complexes are beneficial epistatic interactions; to determine whether these co-adapted gene complexes are important to the organism's fitness, the fitness of early generation hybrid offspring can be examined (Fenster *et al.* 1997;Whitlock *et al.* 1995) (Table 1.1, 1.2). Should the F2 generation exhibit fitness levels below the average of the mid-parent and the F1 generation (MP +F1/2) then this can be taken as evidence of

the disruption of co-adapted gene complexes (Fenster *et al.* 1997; Whitlock *et al.* 1995). Another method of determining whether co-adapted gene complexes are present is to examine the later generation hybrids. After five generations of selfing starting from an F1 which is 100% heterozygous, an organism will be approximately 97% homozygous. Therefore, even if dominance gene effects were contributing to hybrid fitness in the early generation hybrid offspring these dominance effects would no longer be present in the F6 generation. Under simple additivity the trait value of the mid-parent is no different from either the trait value of the F1 or subsequent generations. Therefore, should there be the disruption of co-adapted gene complexes, the later generation hybrids will have a trait value which is less than that of the mid-parent value. These epistatic interactions are more easily found when crosses are performed between distantly related populations or closely related species rather than when crosses are made within populations (Whitlock *et al.* 1995). This is because alleles in one population have usually been selected for to produce a single fitness peak, whereas crosses between populations will result in genotypes which have not undergone selection and will therefore give a better idea of what unselected genotypes would look like (Whitlock *et al.* 1995).

Numerous studies have hypothesized that co-adapted gene complexes at five allozyme loci in *A. barbata* contribute to local adaptation (Clegg and Allard 1972; Allard *et al.* 1972; Allard *et al.* 1993). Although the allelic composition of the Californian and ancestral Spanish gene pools are similar, the actual multi-locus genotypes between these two populations are very different (Garcia *et al.* 1989; Perez de la Vega *et al.* 1991). As the migrant seeds most likely carried a random sample of the Spanish alleles, it would appear that the small amount of outcrossing that occurred in *A. barbata* may have

resulted in allelic combinations which were different from those found in the original Mediterranean population (Perez de la Vega 1991). The subsequent selfing would protect any favourable inter-locus interactions which occurred and selection may have acted on the various allelic combinations to result in one genotype that worked well in the xeric habitat of California and another combination which worked well in the mesic habitat (Perez de la Vega *et al.* 1991). However, as pointed out in Hamrick and Holden (1979), although it would appear that selection acts on these five enzymes, it is unclear whether selection is acting directly on these loci or on some other loci in the genome. It is also possible that if there is a genotype x environment (GxE) interaction for fitness and if one area is colonized predominately by the mesic and xeric ecotypes, then all that is necessary for these patterns of association between genotype and environment to occur is strong directional selection (Hedrick and Holden 1979). If these five loci are in fact working together as a co-adapted gene complex, then the disruption of these loci through hybridization should result in a decrease in fitness of the offspring. However, the same result will be found regardless of whether it is these five loci working together to form a co-adapted gene complex or whether there are other loci elsewhere in the genome which form co-adapted gene complexes.

Most studies that estimate hybrid fitness have been concerned with early generation hybrids; therefore, not only will the study of later, stabilized hybrid generations give a more accurate look at the evolution of hybrids (Rieseberg and Carney 1998), but it will also allow us to determine whether or not the disruption of the putative co-adapted gene complexes and the formation of novel gene complexes in *A. barbata* result in an overall decrease in fitness of the hybrids. By examining the early generation

hybrids we will be able to determine whether additive or dominance gene effects are playing a role in the overall fitness of the hybrids. By examining the later generation hybrids we will be able to determine if there are in fact co-adapted gene complexes which contribute to fitness in *A. barbata*. Also, by examining specific lines within each generation, we will be able to determine if the production of novel gene combinations also results in transgressive phenotypes, which could result in the ability of this species to undergo range expansion.

Methods

Implications of Hybrid Fitness

The genetic basis underlying the fitness of the hybrid offspring should be evident based on the offspring's fitness relative to the parents. One way to determine if epistatic interactions are contributing to fitness is to look at the early generation hybrid offspring (Whitlock *et al.* 1995). If the F2 has a fitness which is lower than the average of the mid-parent and the F1, then this is evidence for the presence of epistasis (Whitlock *et al.* 1995). Fenster and Galloway (2000b) used this approach to document epistatic effects in *Chamaecrista fasciculata.* Due to difficulties in creating the F1 generation in *A. barbata*, relatively few were obtained and this generation was not included in this study. Instead, the F2 generation was used to represent the early generation of hybridization. As the mesic and xeric ecotypes were homozygous, and fixed for opposite alleles at various loci, the F2 generation should be approximately 50% heterozygous. If the F2 generation displays on average fitness level no different from the mid-parent then there will be no evidence for dominance or epistatic gene effects and the underlying genetic basis can be assumed to be additive. If however, the fitness levels of the F2 are significantly higher than the mid-parent then this is evidence for the presence of heterosis, which may be due to dominance, overdominance or epistasis (Stuber *et al.* 1992; Mitchell-Olds 1995). Should there be evidence for heterosis in the early generations, and if the heterosis is due to dominance or overdominance, then the fitness of the later generations hybrids (i.e. F6) should be no different than the mid-parent assuming no epistatic interactions. After five generations of selfing individuals should be approximately 97% homozygous. Thus, any dominance effect which may have been present in earlier generations would no longer be

present. Therefore, if the F6 generation exhibits fitness levels which are significantly different from the mid-parent this may be taken as evidence for the presence of epistasis. Although hybrid breakdown may be occurring in a particular group of hybrids, resulting in a low average fitness of the recombinants, some individual genotypes within this group may be as fit or more fit than the parental genotype (Rieseberg and Carney 1998).

Study Design

F2 and F6 hybrid families were produced by an initial crossing of a mesic and xeric ecotype followed by selfing to the F6 generation (Figure 1.1). After the initial out-crossing event, which resulted in completely heterozygous F1 offspring, the plants were allowed to self fertilize. As heterozygosity is decreased by half with each generation of selfing then after five generations of selfing the individual plants should be 97% homozygous. This means that each line of the F6 generation is a stabilized, true breeding hybrid derivative. Twenty five of the F6 families were randomly chosen to be used in this experiment.

A total of 50 mesic seeds along with 50 xeric, 100 F2 seeds (50 from family F2-A, 10 from each of family F2-B,C,D,E,F), and 400 F6 seeds (16 seeds from each of 25 families) were planted in July 2001 in the Dalhousie University greenhouse. The seeds were germinated by placing them in petri dishes lined with wet filter paper. The petri dishes were placed in the refrigerator for three days, at which point they were removed and allowed to germinate in the dark at room temperature. Seeds were planted in pots containing a mixture of 1/3 organic black earth, 1/3 sand and 1/3 peat moss. A randomized complete block design was used with the pots randomized across three blocks with 200 plants per block and all families represented in each of the three blocks.

The plants were watered as needed and fertilized every two weeks using Plant Prod 15:15:18 (Plant Products Ltd. Brampton Ontario). Flowering time, measured as the emergence of the awns from the first panicle, was recorded throughout the experiment. Day one of flowering time was the first day that flowering occurred. After 7 months, which is approximately the lifespan of *A. barbata* in the wild, the plants had reached senescence. At this point fitness, which was quantified as the number of spikelets produced per plant, was recorded. *A. barbata* is an annual plant which means the number of spikelets produced is a measure of the individual plant's total reproductive output over its lifetime. Individuals which had not reproduced at the time of harvest were assigned a fitness of zero. The plants were cut off at soil level, placed in paper bags and dried at 55°C for two weeks at which point dry weights were obtained.

Statistical Analysis

All analyses were carried out using SAS Proc GLM unless otherwise stated. As only 29 of the 600 plants used in this study did not survive and there was no particular family which seemed to have a higher rate of survivorship than any other family those plants which did not survive were coded as missing values, while those plants which had not produced spikelets by the end of the experiment were assigned a fitness value of zero. This distinction between those plants which did not survive and those which survived yet did not reproduce was made as there was no selection on survivorship in the greenhouse and the death of individual plants was random. Analysis of variance (ANOVA) was carried out on fitness, flowering time and above ground biomass to ascertain if there was a significant difference in the long term (F6 generation) versus the short term (F2 generation) effects of hybridization. Block, generation and family (nested within

generation) were included as main effects in all models. Block and family were considered random effects and fitness was analysed both with and without flowering time as a covariate. Interaction terms were tested (block x generation and block x family) and if they were found to be non-significant they were removed from the analyses. Linear contrasts were performed to compare the average F2 and F6 fitness to the mid-parent fitness. To determine if any of the family means differed significantly from the mid-parent value a Dunnett's test was performed. This procedure tests whether the mean of a control group (in this case the mid-parent) differs significantly from the mean of the other groups (the families) (Zar 1999). This test was also used to determine if the performance of any of the hybrid families was significantly different from either the mean mesic or mean xeric trait value. Variation among families in survivorship was tested using RXC, a programme which calculates the chi-square and G-statistic of a 2-way contingency table (Roff and Bentzen 1989). To determine if either flowering time or biomass were related to fitness, correlations of the family means were calculated using Pearson's correlation coefficient in SPSS.

The additive, dominance and epistatic effects were calculated for the three traits studied using the expectations for each generation as found in Mather and Jinks (1982) (Table 1.1). The additive effect was calculated as half the difference between the mesic and xeric means. The dominance effect was calculated as twice the difference between the F2 and F6 generations, and the epistatic effect was calculated as the difference between the F6 generation and the mid-parent (Table 2.1). For simplicity the term DxD was assumed to be zero. The 95% confidence limits for differences between two means were calculated according to Sokal and Rohlf (1995). Should the confidence limits

Table 2.1: Contrasts used to calculate the additive, dominance and epistatic gene effects. The contrasts are made using the expectations for generation means as given in Table 1.1.

Contrast	Expected Generation Means (Table 1.1)	Interpretation
P1-P2	(m + A + AxA) – (m – A + AxA)	2A
F2-MP	(m + 1/2D + 1/4DxD) – (m + AxA)	1/2D + 1/4DxD - AxA
MP-F6	(m + AxA) – m	AxA
F2-F6	(m + 1/2D + 1/4DxD) - m	1/2D + 1/4 DxD

include the zero point it was assumed there was no significant difference between the two means and thus the effect size was not significant.

Results

No significant differences in survivorship were found between families ($p=0.07$). As the interaction terms were non-significant they were not included in the analyses presented here. Significant family effects were found for fitness, flowering time and biomass. Linear contrasts between the F2 generation and the parental generation for all three traits were found to be non-significant. Linear contrasts for the F6 generation vs. parents as well as the F6 vs. F2 were significant for fitness and flowering time (Table 2.2,2.3), however, only the contrast between the F6 generation and the parents was significantly different for above ground biomass (Table 2.4).

The effect sizes of additive, dominance or epistatic gene effects were calculated using the expectations for generation means (Mather and Jinks 1982). It was found that there were significant additive, dominance and epistatic effects for fitness and flowering time; however, only the dominance and epistatic effects were significant for above ground biomass. Dominance effects were found to be larger than either the additive or epistatic effects for all three traits and the additive effects were larger than the epistatic effects for fitness and flowering time (Table 2.5).

Fitness and flowering time were significantly correlated ($r=-0.899$, $p<0.01$) (Figure 2.1). Flowering time was used as a covariate of fitness in the analysis to determine if the difference in fitness among families was simply a function of differences in flowering time (Table 2.6). Nevertheless, even with flowering time included as a covariate, family effects were still found to be significant. This indicates that not all the fitness variation among families is accounted for by flowering time. Fitness and biomass were not correlated ($r=-0.147$, $p=0.415$) (Figure 2.2).

Table 2.2: Analysis of variance on fitness (spikelet number) to evaluate effects of long term and short term hybridization in *Avena barbata*. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	14874	6.84**
Generation	2	12581.13	0.47
Family within generation	30	16518	7.6****
Error	535	2173.53	
Linear Contrasts			
Parents vs. F2	1	59.03	0.03
F2 vs. F6	1	14699.55	6.62*
Parents vs. F6	1	16726.88	7.54**
Error	534	2218.87	

* P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 2.3: Analysis of variance on flowering time to evaluate effects of long term and short term hybridization in *Avena barbata*. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	1690.36	2.52
Generation	2	9184.82	1.16
Family within generation	30	4829.08	7.19****
Error	499	672.03	
Linear Contrasts			
Parents vs. F2	1	259.92	0.40
F2 vs. F6	1	11654.92	17.80****
Parents vs. F6	1	11406.76	17.42****
Error	503	654.91	

* P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 2.4: Analysis of variance on above ground biomass to evaluate effects of long term and short term hybridization in *Avena barbata*. Linear contrast were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	166.15	53.14****
Generation	2	13.23	0.76
Family within generation	30	11.56	3.70****
Error	534	3.12	
Linear Contrasts			
Parents vs. F2	1	112.65	2.50
F2 vs. F6	1	0.20	0.00
Parents vs. F6	1	200.92	4.46*
Error	532	45.08	

P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 2.5: Ecotype and generation means ± SE. Additive, dominance, and epistatic effects of fitness, flowering time and above ground biomass calculated using expectations for generation means as found in Mather and Jinks (1982). 95% confidence limits for differences between two means were calculated according to Sokal and Rohlf (1995).

	Fitness	Flowering Time	Above Ground Biomass
Mesic Mean	94.47 ± 6.75	44.44 ± 3.09	9.25 ± 0.3
Xeric Mean	33.45 ± 6.83	69.6 ± 2.59	9.46 ± 0.29
F2 Mean	70.28 ± 4.92	52.61 ± 2.53	9.1 ± 0.18
F6 Mean	50.62 ± 2.82	67.57 ± 1.66	8.79 ± 0.11
Additive Effect	30.5 ± 20.05	12.03 ± 10.98	0.1 ± 0.83
Dominance Effect	41.95 ± 11.13	31.25 ± 6.28	0.69 ± 0.42
Epistatic Effect	14.69 ± 4.41	11.071 ± 2.16	0.6 ± 0.20

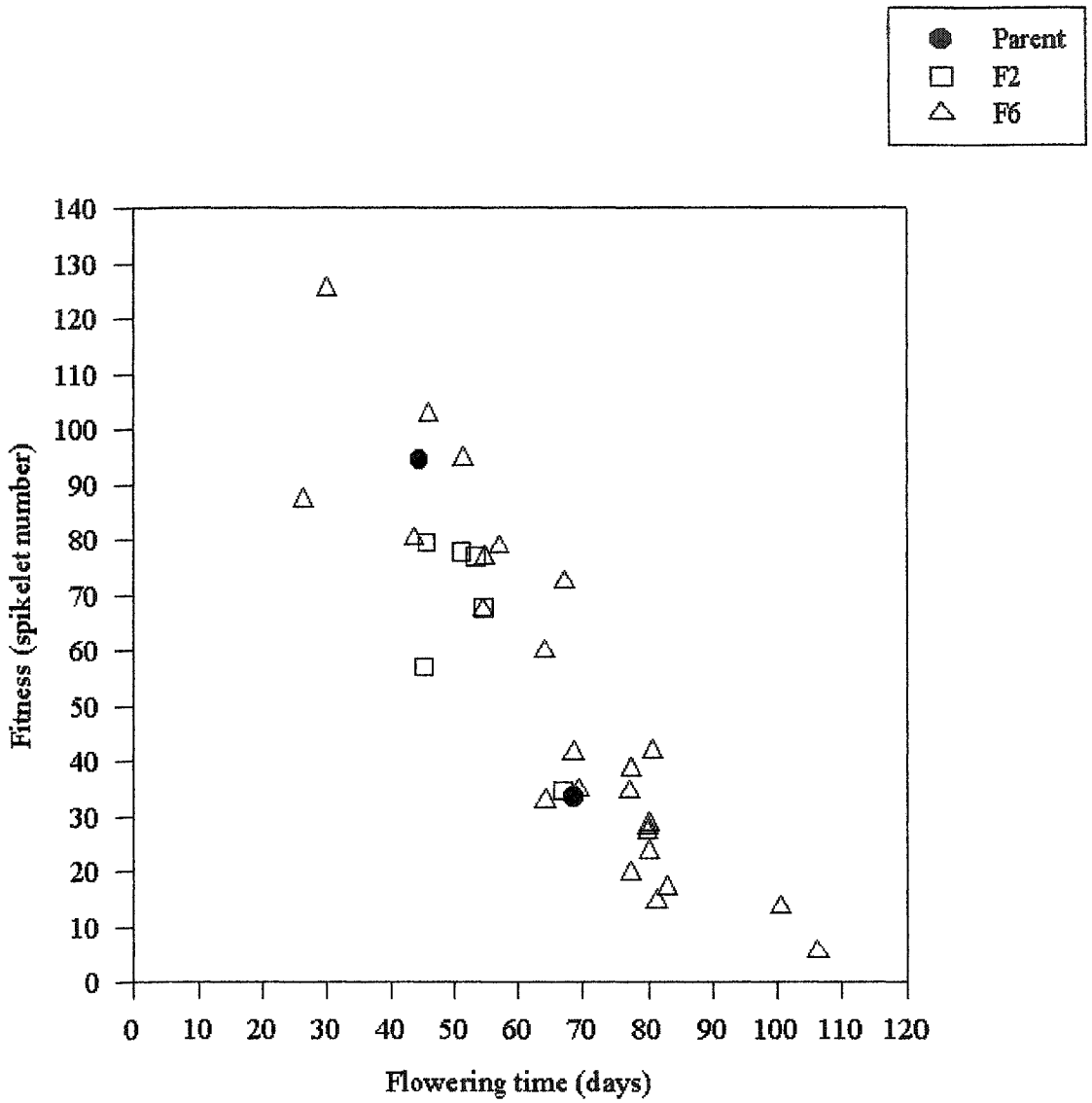

Figure 2.1: Mean fitness of each family vs. mean flowering time of each family for mesic and xeric ecotypes, six F2 families and 25 F6 families of *A barbata*. Correlation coefficient = -0.899

Table 2.6: Analysis of variance on fitness (spikelet number) with flowering time as covariate.

Source	df	Mean Square	F value
Block	2	6351.38	4.06*
Flowering time	1	319167.24	204.06****
Generation	2	118.57	0.02
Family	30	4777.49	3.05****
Error	498	1564.11	

* P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

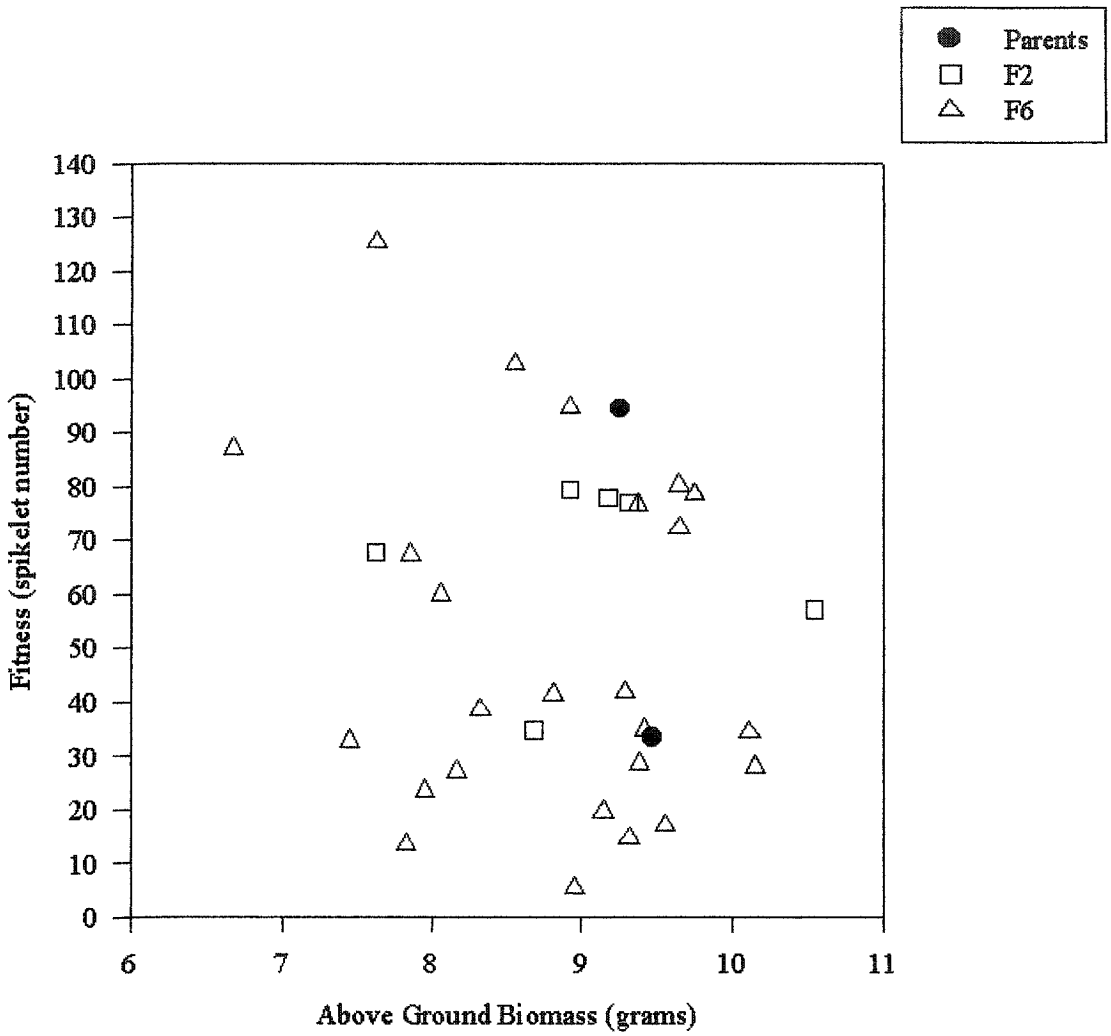

Figure 2.2: Mean fitness of each family vs. mean above ground biomass of each family for mesic and xeric ecotypes, six F2 families and 25 F6 families of *A. barbata*.

Correlation coefficient = -0.129

To ascertain which specific families were different from the mid-parent value the Dunnett's test was performed. It was found that seven F6 families had fitness's significantly worse than the mid-parent and two F6 families had fitness's significantly better than the mid-parent (Figure 2.3). Eight families flowered significantly later and two families significantly earlier than the mid-parent (Figure 2.4); and four families were found to have a significantly lower biomass than the mid-parent (Figure 2.5). The families are arranged in the same order in all three of these figures for comparison.

Evidence for transgressive segregation in the F6 generation was found by using the Dunnett's test and testing the family means against the mesic and xeric means separately. There were ten families which had a fitness lower than the xeric ecotype and three families with a higher fitness than the mesic ecotype, although none were significantly higher than the mesic ecotype or significantly lower than the xeric ecotype. In terms of flowering time, two families flowered significantly later than the xeric plants, which is evidence for transgressive segregation. Eleven other families flowered later than the xeric ecotype and three families flowered earlier than the mesic ecotype although these were not significant. Three families were significantly lower in biomass than the mean mesic ecotype, again showing evidence for transgressive segregation. Fifteen other families were also lower in biomass than the mesic ecotype, while seven families had a greater biomass than the xeric ecotype. Again, these were not significant.

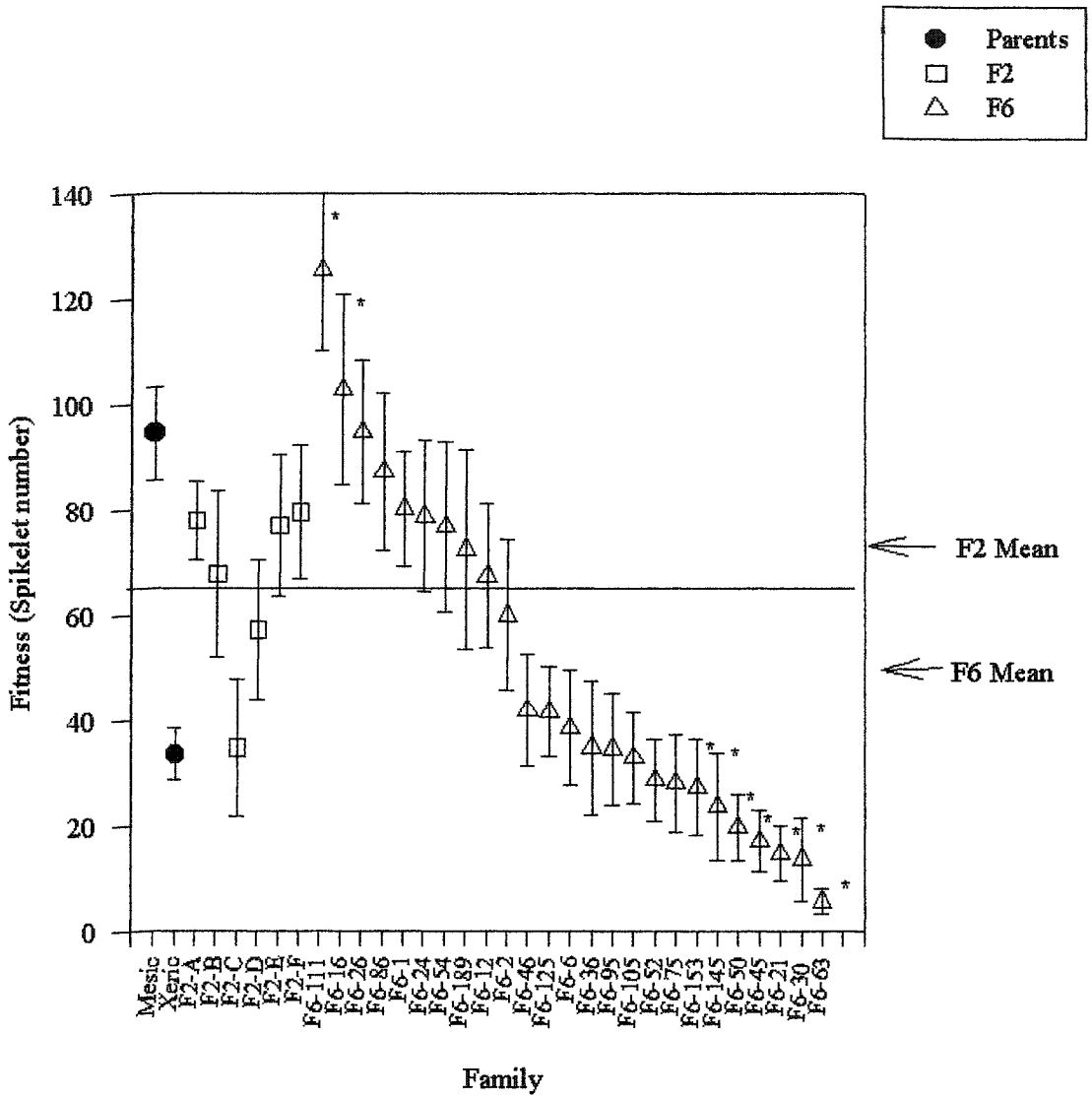

Figure 2.3: Mean fitness (spikelet number) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families of *A. barbata*.

*=significantly different from mid-parent value

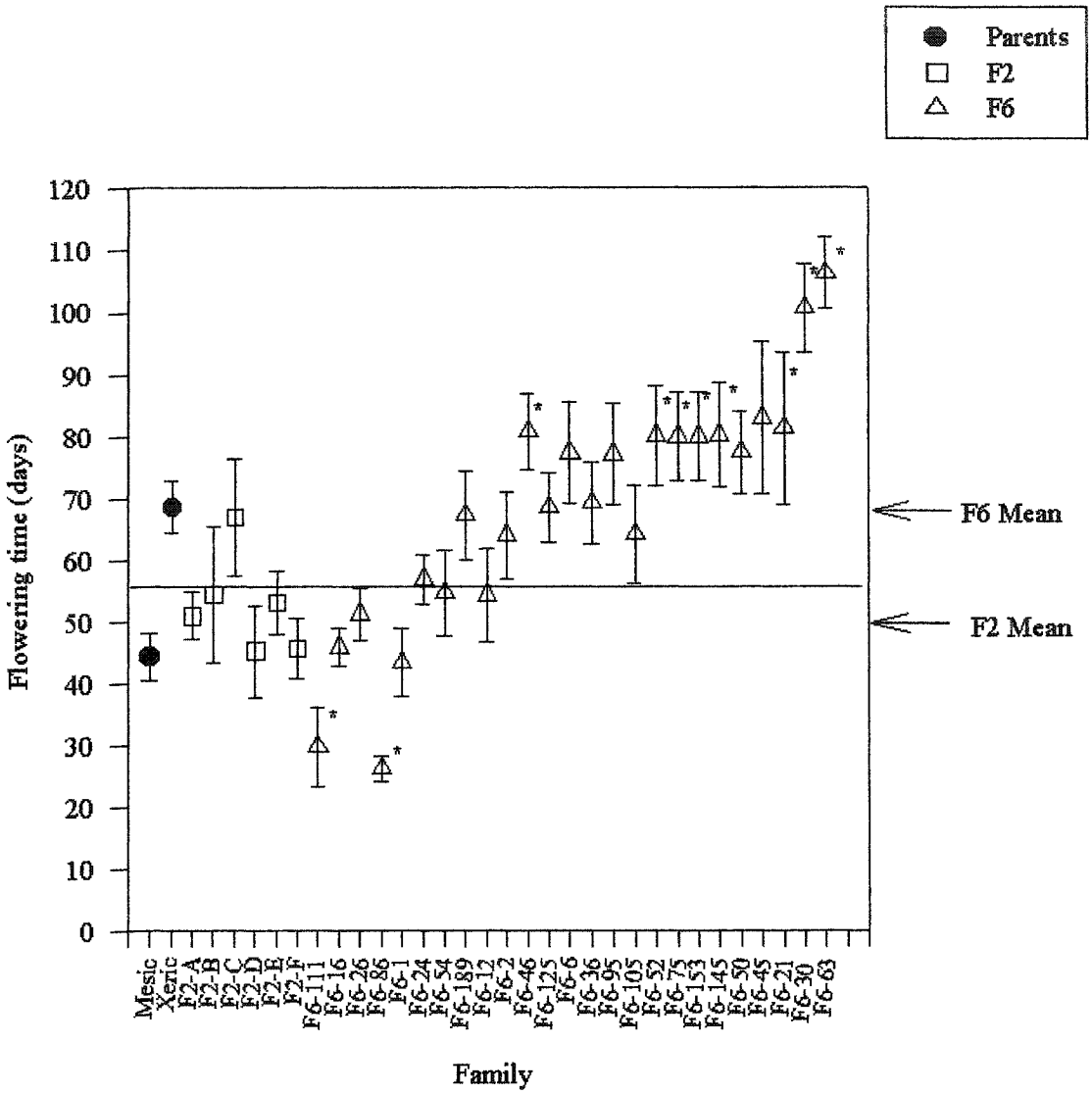

Figure 2.4: Mean flowering time (days) ± standard error (day 1 = first day flowering occurred) of mesic and xeric ecotypes, six F2 families and 25 F6 families of *A. barbata*. *= significantly different from mid-parent value.

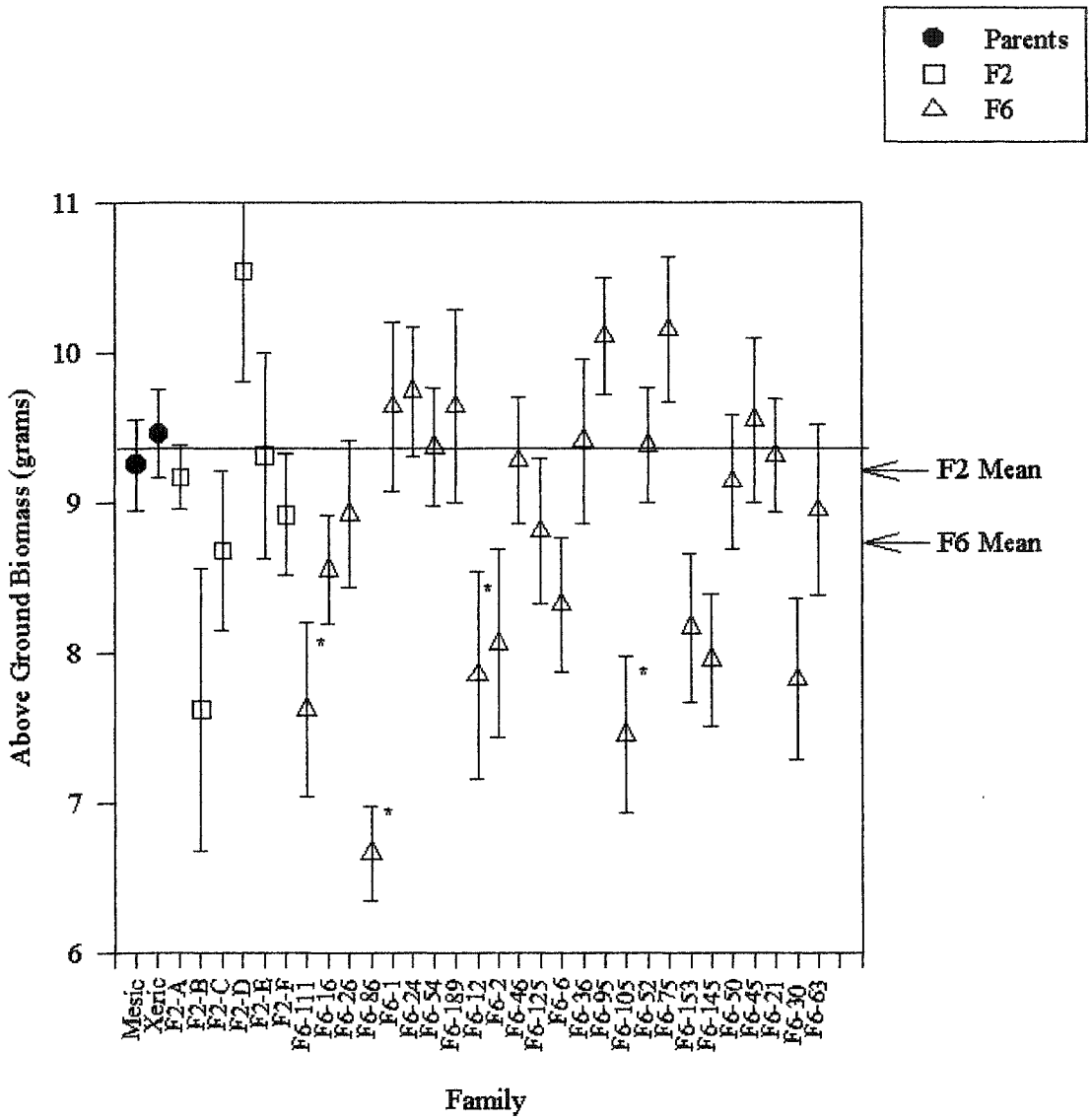

Figure 2.5: Mean above ground biomass (grams) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families of *A. barbata*.

*= significantly different from mid-parent value.

Discussion

The primary purpose of this study was to determine what, if any, fitness differences exist between early and late generation hybrids and their parents. The absence of any significant differences between generations in the ANOVA (Tables 2.2, 2.3, 2.4) seems to indicate that the genetic effects are simple additive effects with no heterosis or hybrid breakdown. However, both the linear contrasts that were performed and the calculation of the net effect of additive, dominance and epistatic interactions indicate that there is a difference between the F6 generation and both the parental and F2 generation, for fitness and flowering time, and between the F6 generation and the parental generation, for above ground biomass. This inconsistency may be in part due to the relatively few degrees of freedom available in the ANOVA for testing the difference among generations. By including family in the ANOVA, some of the power is taken away from testing the difference among generations. As family is nested within generations, the generation term must be tested over the family term which means there are 2 degrees of freedom in the numerator but only 30 degrees of freedom in the denominator. Where the testing of generations in the ANOVA tests the hypothesis that parents=F2=F6, the linear contrasts between generations concentrate on detecting differences between specific groups. This results in an increase in the power to detect differences as, although there is only 1 degree of freedom in the numerator, there are over 500 degrees of freedom in the denominator for all three traits. As the question of interest is not simply whether parents=F2=F6 but rather whether specific generations are different from each other the discussion will focus on the linear contrasts rather than the main effect of generation in the ANOVA. By focussing on the linear contrasts rather than the

results from the ANOVA it is possible to determine whether additive, dominant or epistatic gene effects are contributing to the generation means and thus whether there is any hybrid breakdown or hybrid vigour occurring. As there are three linear contrasts yet only 2 degrees of freedom for the main effect of generation in the ANOVA this means that there are only two independent contrasts which may be carried out. This may mean that one of the contrasts may result in a Type II error, however, these types of contrasts are legitimate if the contrasts to be performed were decided upon a priori. Performing a Tukey's test will result in a more conservative estimate of which contrasts are significantly different from one another, and although not shown here, Tukey's test does result in the same outcome as the linear contrasts.

The lack of significant differences between the F2 generation and the parental generation for all three traits studied indicates that there was no hybrid vigour present in the early generation hybrids but that neither was there any hybrid breakdown. However, the linear contrasts combined with the significant dominance and epistatic effects which were found when the net effect of additive, dominance and epistatic interactions were calculated indicate that simple additive gene effects are not all that is contributing to fitness and flowering time. The comparison between the F2 generation and the parental generation tests for presence of dominance as well as both dominance x dominance epistasis and additive x additive epistasis (Table 2.1). If the average of the F2 generation is no different from that of the mid-parent then there is no evidence for either dominance or epistatic gene effects, which is consistent with additive gene effects. However, if the underlying genetic basis of both hybrid fitness and flowering time is simply due to additive gene effects then the fitness of the F6 generation should also be no different

from the mid-parent or the F2 generation. Any deviation of the F6 mean value from the mid-parent value is evidence for the presence of epistasis, while deviation from the F2 value is evidence for the presence of dominance. As linear contrasts between both the F2 and F6 generations and between the F6 and parental generations for fitness and flowering time were significant this would seem to indicate that there is in fact some dominance gene effects in the F2 generation. However, the break-up of co-adapted gene complexes is counteracting any increase in fitness or flowering time in the early generation hybrids. The loss of heterozygosity in the later generation means that any dominance gene effects which were originally present are no longer present; therefore, the loss of fitness and the later flowering times seen in this generation must be the result of the break-up of co-adapted gene complexes.

Above ground biomass behaved in a slightly different manner with the only significant difference between generations being between the F6 and the mid-parent. This indicates there is some break-up of co-adapted gene complexes in the F6 generation while the F2 generation does not appear to be affected by either dominance or epistasis. However, although the dominance effect for above ground biomass is again the biggest effect this does not seem to make a significant difference to the F2 fitness. If dominance gene effects were playing a large part in the mass of the plants, then there should have been a difference in mass between the F2 plants and the F6 plants, which was not the case. However, it is possible that even if there are dominance gene effects playing a role in the biomass of the plants, the differences among generations for above ground biomass are so small that differences between the F2 and the other two generations may be hard to detect.

The large amount of dominance gene effects found is interesting as it has been suggested that heterosis will be low in populations in which there is strong selection against deleterious alleles and with low migration rates (Whitlock *et al.* 2000). Instead, heterosis will be greatest when there is a high mutation rate combined with intermediate levels of selection, which allows for the allele frequencies to diverge to such an extent that heterosis will be noticeable (Whitlock *et al.* 2000). As the two ecotypes outcross no more than 5% of the time it can be assumed that the migration rate between the populations is low. However, as the two ecotypes of *A. barbata* are hypothesized to be locally adapted to their environments, one would assume that selection has acted to create the most fit genotype for that environment and that there are few deleterious alleles. It is possible that the deleterious alleles are of small effect with only intermediate selection against them and this, combined with the low migration rate will result in the two ecotypes having slightly different deleterious alleles. Not only would the two ecotypes of *A. barbata* have to have different deleterious alleles, but as this is a selfing species and is assumed to be homozygous at most loci, the deleterious alleles would most likely be fixed within each ecotype. The combination of deleterious alleles which undergo moderate levels of selection and the fixing of the different deleterious alleles within each ecotype will allow for the masking of these recessive alleles in the hybrids and thus a large dominance effect resulting in hybrid vigour (Whitlock *et al.* 2000).

While fitness and flowering time are strongly correlated, indicating that the high fitness exhibited by some families may simply be due to the earlier flowering time rather than due to any gene effects acting on fitness independent of flowering time, there was still a significant difference among families when flowering time was included in an

ANOVA as a covariate (Table 2.5). This is consistent with flowering time playing a part in the fitness of the individual families. However, as not all the variation among families can be explained by flowering time it is possible that other traits apart from flowering time may also be affecting the fitness of the hybrids. The recombination which took place results in novel gene combinations in the hybrid offspring. While the mean of the F6 generation seems to be lower than the mid-parent value for fitness and later than the mid-parent value for flowering time, families which are significantly different from the mid-parent value for both fitness and flowering time are present. This indicates that the novel gene combinations in some recombinant families may potentially allow for the colonization of novel environments.

Previous studies have shown there to be a correlation between above ground biomass and spikelet number. This correlation may be in the form of a negative relationship, indicating a trade off between allocation of resources, or a positive relationship, indicating the ability of larger plants to better utilize the resources and thus produce more seeds. Whether there is a correlation between plant size and fecundity produced depends on the species in question. Aarssen and Clauss (1992) and Watkinson (1982) both found that as size increases so does seed number, however Aarssen and Clauss (1992) found that a plant which produces a large number of seeds when large, does not necessarily produce a large number of seeds when small. Rees and Crawley (1989) found that the relationship depended on the species, with a negative relationship occurring only when the reproductive unit was large. However, under greenhouse conditions there does not appear to be any relationship between above ground biomass and spikelet number in *A. barbata* indicating that a high biomass did not necessarily

result in a high fitness. Whether the same result would occur in the field where the plants have to compete for resources, will be tested in a subsequent experiment (Chapter 4).

Transgressive segregation is the formation of extreme phenotypes, relative to either of the parents (deVicente and Tanksley 1993; Rieseberg *et al.* 1999; Rieseberg *et al.* 2003). Rieseberg *et al.* (1999) state that a trait in hybrid offspring may be transgressive if it falls outside the parental range in either direction, regardless of whether the extreme trait value is significantly different from the parental value. Previous studies have looked at transgressive segregation in early generation hybrids (deVicente and Tanksley 1993; Lexer *et al.* 2003a). A study which examined the BC2 generation between *H. annuus* and *H. petiolaris* found that while transgressive segregation was present for some BC2 individuals within the segregating hybrid population, mean fitness was intermediate to the parents (Lexer *et al.* 2003a). A similar result was found in the deVicente and Tanksely (1993) study in which *Lycopersicon esculentum* and *L. pennellii* were crossed and some extreme individuals in the F2 population were found. Although none of the F6 families were found to exhibit fitness values which were significantly better or worse than the most fit or least fit parent, the fact that entire families rather than a few individuals do fall outside the parental range supports the theory that there is transgressive segregation in the F6 generation. Entire families which fall outside the parental range is consistent with the family trait value being due to a genetic effect rather than simply an environmental one, which might be the case if only an individual plant fell outside the parental range. Similar results are found for flowering time, which, given the strong correlation between these two traits is not surprising, although, in the case of flowering time, there were two families that flowered significantly later than the xeric

ecotype. One family (F6-86), which exhibits a much earlier flowering time than the mesic parent, does not have an extreme fitness value. This was to be expected in some families as, despite the correlation between flowering time and fitness, fitness values can not be explained entirely by flowering time. Above ground biomass appears to exhibit a great deal of transgressive segregation. The mean values for the mesic and xeric parents are not much different (9.26 grams vs. 9.46 grams) from each other. Rather than resulting in a very narrow range of weights for the offspring, this instead resulted in a large number of transgressive phenotypes, with three families weighing significantly less than the mesic parent and twenty-two other families falling outside the parental range.

Whether the transgressive segregation in *A. barbata* is caused by complementary gene action or epistatic interactions can not be determined by this study, although a QTL study by K.M. Gardner (personal communication) did find a number of complementary genes in the two ecotypes. Transgressive segregation has usually been attributed to complementary gene action (Rieseberg *et al.* 2003). If this is the case in *A. barbata* then it would appear that not all of the plus or minus alleles are found in one of the parental ecotypes (i.e. all the plus alleles in the mesic ecotype) but rather both plus and minus alleles must occur in both the mesic and the xeric ecotypes. It has been suggested that the production of extreme phenotypes such as these may contribute to adaptation and speciation (Lexer *et al.* 2003a; Rieseberg *et al.* 2003). Determining whether the extreme phenotypes produced in *A. barbata* will result in an advantage in novel environments is the next step in determining if hybridization of these two ecotypes can result in adaptation to a non-parental environment.

Frequently, hybrids resulting from a cross between two particular species or ecotypes are regarded as one homogeneous group and discussion centres around whether the group as a whole is more or less fit than the parental species. This in turn leads to discussions of whether the hybrid group as a whole is bad (the hybrids are inviable or sterile) (Wagner 1970; Mayr 1992) or good (the hybrids may exhibit hybrid vigour and outperform the parents under certain situations) (Moore 1977; Arnold 1997), as well as to discussions about what model of hybrid zone best fits the outcome of the hybridization event. However, while the generation mean of the hybrid offspring may be different from the parental mean, the presence of transgressive segregation may result in a particular genotype which is capable of outperforming the parent in a certain environment, whether it is the parental environment or a novel environment. If the loss of fitness in the F6 generation were considered on its own without looking for the presence of transgressive segregation, it would lead to the conclusion that hybrids between the two ecotypes of *A. barbata* are less fit than the parental ecotypes and therefore would not contribute to the evolution of the species. However, by looking for transgressive segregation it becomes clear that while on the whole the later generation hybrids do have a lower fitness than the parents, there are individual genotypes that are capable of outperforming the parental ecotypes. So, while certain genotypes may in fact be inviable, sterile or simply less fit than the parental ecotypes and may not contribute to the evolution of the species, other genotypes may in fact be able to outperform the parents which in turn may lead to adaptations to novel environments. Thus, when examining whether hybridization will lead to range expansion and speciation or whether it is an evolutionary dead end, it is

perhaps more accurate to look at the outcome based on individual hybrid genotypes rather then on the generation mean as a whole.

Chapter 3

Fitness of Mesic, Xeric and F6-generation hybrids of *Avena barbata* in novel environments

Introduction

Hybridization between two differentially adapted populations and the subsequent recombination that occurs in the F2 generation and beyond may result in increased genetic variation (Ellstrand and Schierenbeck 2000). While this increased variation does not always result in hybrid offspring with fitness superior to that of the parents, and may in fact result in hybrids which are much less fit, it is possible that novel gene combinations which result in adaptation to a new environment may be formed (Ellstrand and Schierenbeck 2000; Hochwender *et al.* 2000). If the hybrid offspring spread to new environments and are capable of outperforming the parents, this may lead to successful colonization of new territories.

Genotype-by-environment (GxE) interactions have been reported for a number of species (Bell 1991; Hatfield and Schluter 1999; Fritsche and Kaltz 2000; Welch and Rieseberg 2002). As discussed in Chapter 1, numerous species exhibit GxE interactions including *A. tridentata* ssp. *tridentata* and *A. tridentata* ssp. *vaseyana* and their hybrids (Wang *et al* 1997), *G. capitata* ssp. *capitata* and *G. capitata* ssp. *chamissonis* (Nagy and Rice 1997), *I brevicaulis* and *I. fulva* and their hybrids (Johnston et al. 2001a) as well as the limnetic and benthic forms of sticklebacks and their F1 hybrids (Schluter 1995; Hatfield and Schluter 1999). Studies that examined the fitness of hybrids, both inside an established hybrid zone as well as in the parental environments, have documented that the relative fitness of the hybrid is not necessarily independent of the environment (Emms

and Arnold 1997; Wang *et al.* 1997; Campbell and Waser 2001) but instead depends on both the interactions between the parental genomes and the interactions between the genotypes and the environment (Bordenstein and Drapeau 2001). Examination of the reaction norms, which are a genotypes phenotypic response across different environments, will help to determine if GxE interactions are occurring. If there are no interactions between the genotypes and the environment then the hybrid genotypes may show similar responses to different environments, which would result in parallel reactions norms (Bordenstein and Drapeau 2001). Alternatively, if GxE interactions are occurring, there will be different responses by the various genotypes to changing environments, which would result in non-parallel reaction norms (Bordenstein and Drapeau 2001).

The overall outcome of hybridization will be affected by whether there are interactions between parental genomes (endogenous selection) or between the hybrid genotype and the environment (exogenous selection). The use of recombinant hybrids makes it possible to study whether the parental gene combinations are the best combinations or whether hybridization can lead to novel beneficial combinations. If the initial hybridization event is followed by self fertilization then any advantageous novel combinations may remain as beneficial epistatic combinations that confer adaptations to certain environments (Allard 1996). Also, through complementary gene action recombination may lead to the formation of individuals with transgressive phenotypes, these individuals may be better adapted than the parents to novel environments. Alternatively, negative interactions between parental genomes may occur when certain alleles, which work well in one genetic background, interact epistatically with another genetic background leading to loss of fitness and possibly sterility or inviability

(Bordenstein and Drapeau 2001). This in turn will prevent the hybrid offspring from expanding into a new environment or out-competing the parent in the parental environment. Asymmetric interactions may also occur whereby the hybrid fitness will depend on the direction of the cross. These interactions between the parental genomes may be environment dependent; for example Campbell and Waser (2001) found that asymmetry in the fitness of the F1 hybrid offspring of *Ipomopsis aggregata* and *I. tenuituba* was dependent on the environment that the hybrids were growing in. Hybrids that had *I. aggregata* as the maternal parent exhibited a greater fitness than those that had *I. tenuituba* as the maternal parent at both the parental sites, as well as outperforming both parents at the *I. aggregata* site. However, those hybrids which had *I. tenuituba* as the maternal parent exhibited a higher fitness than those that had *I. aggregata* as the maternal parent at the hybrid site (Campbell and Waser 2001).

Very few studies have used hybrids of known origin rather than natural hybrids of an unknown genetic background to examine GxE interactions and what effect these interactions may have on fitness (Campbell and Waser 2001). The pattern of association found in the Californian population of *A. barbata* between allozyme frequencies and the environment is found on both a macro and micro geographical scale (Allard *et al.* 1972). Migration of this species within California allowed for different genotypes to be tested in different environments (Clegg and Allard 1972). It is thought that the genotype which was most adapted to a particular environment was selected for, resulting in a mesic genotype adapted to a moist environment and a xeric genotype adapted to a more arid environment (Clegg and Allard 1972). If these genotypes contain co-adapted gene complexes as suggested by the results in Chapter 2, then the hybrid offspring should have

a lower fitness in the parental environment (this will be tested in Chapter 4). By the same reasoning, it also seems likely that the parents should have a low fitness in novel environments as they appear to be locally adapted to a particular environment in California. The hybrid families that were produced have novel genotypes which may allow them to do well in a non-parental environment. It may be that one particular genotype does well in one environment but not in others, indicating the possibility of a new gene combination conferring adaptation to a particular novel environment. Alternatively, it may be that one genotype, whether it is a parental or a novel genotype, may do well over several environments, thereby allowing that particular family to adapt to a broad range of different environmental conditions.

Methods

This experiment examined twelve F6 families in four different environments to ascertain if GxE interactions occur and if any of these families outperform the parental ecotypes in any one environment. Twelve of the twenty-five F6 families used in experiment one were chosen to be used in this experiment. The twelve families were chosen to represent the entire range of fitness values found in Chapter 2, with four families representing high fitness values, four representing low fitness values and four representing those families with fitness values around the average for the F6 generation.

Seeds were germinated by placing them in petri dishes lined with wet filter paper. The petri dishes were placed in the refrigerator for three days at which point they were removed and allowed to germinate in the dark at room temperature. After three days the seeds had germinated; they were then planted in cell packs and placed in a growth chamber with a twelve hour light/dark cycle. The temperature within the growth chamber was 20°C during the light cycle and 15°C during the dark cycle. After two weeks the plants were transferred to pots in the greenhouse and the pots were placed in trays with eight pots per tray. The plants were allowed to become established in the pots for two weeks at which point the environmental regimes were initiated.

Eighty mesic seeds, eighty xeric seeds and sixty-four seeds from each of the twelve families were divided equally among the eight blocks and their position within each block was randomized. Two blocks were assigned to each of four environments, made up of all four combinations of two different watering regimes and two different fertilization regimes (Figure 3.1). The placement of the environments within the greenhouse was random. The high water treatment was watered every other day and

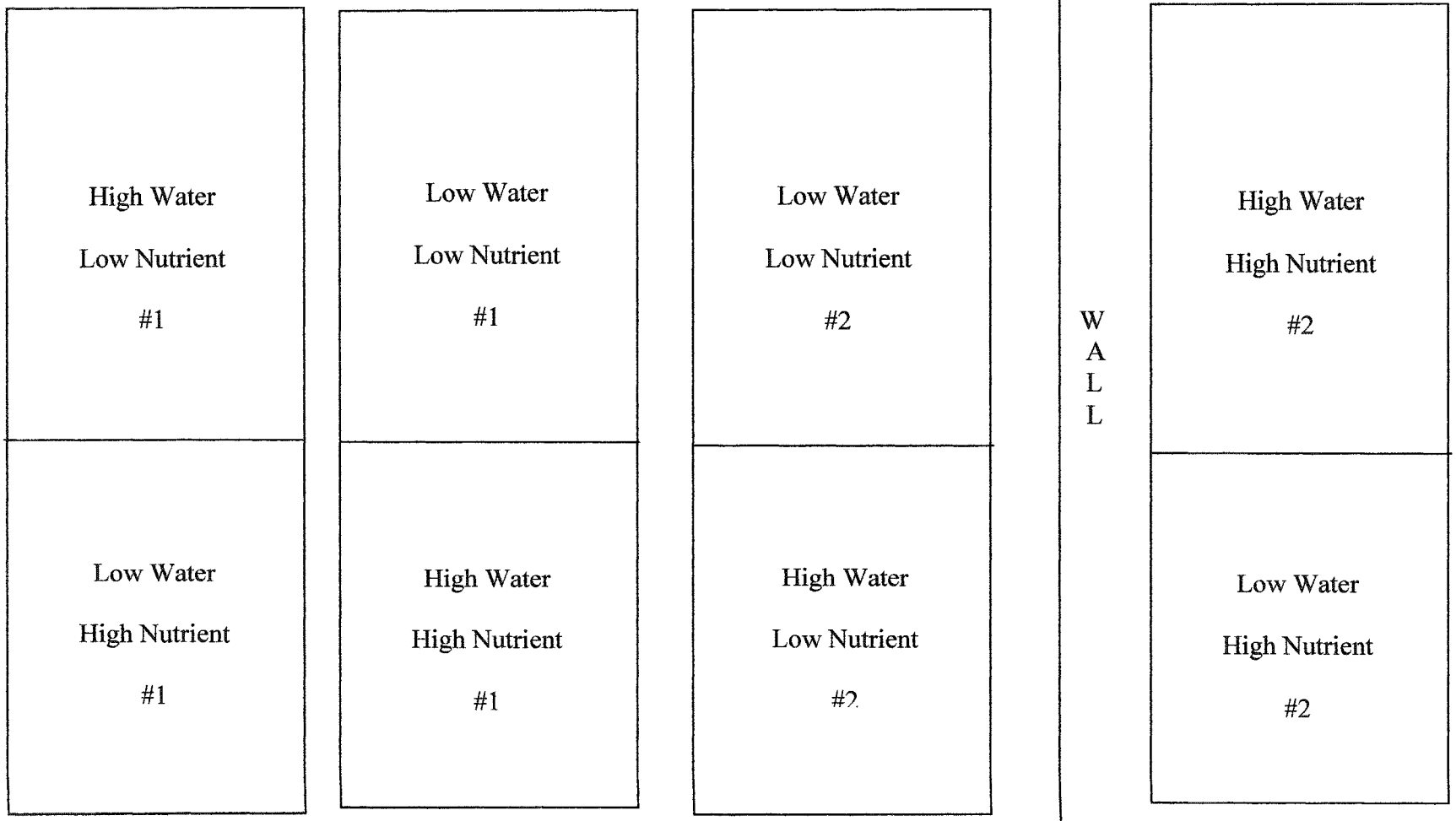

Figure 3.1: Placement of environments within Dalhousie University Greenhouse.

the low water treatment once a week, except during the extremely hot weather of July and August (when it received water twice a week). The low water treatment was altered for July and August due to the high temperature in the greenhouse, which would have resulted in the death of half the plants without an additional watering. The high nutrient treatment was fertilized once a week and the low nutrient treatment received fertilizer once during the course of the experiment. The fertilizer used contained nitrogen, phosphorous and potassium at a ratio of 15:15:18 (Plant Products Ltd. Brampton Ontario) and was added directly to the water which was used for the watering regime. The plants were watered by adding water to the trays which was then absorbed by the soil in the pots until fully saturated.

Flowering time was recorded throughout the experiment. After seven and a half months the experiment was halted, the spikelet number recorded and the above ground portion of the plant collected for dry weight measurements in the same manner as in Chapter 2.

Statistical Analysis

Unless otherwise stated all statistical analyses were performed using SAS Proc GLM. As in Chapter 2 plants as only 39 of the 928 plants used in the experiment did not survive and as survivorship between families did not appear to be different the plants which did not survive were coded as missing values, while those plants which had not produced spikelets by the end of the experiment were assigned a fitness value of zero. Analysis of variance was performed first on the full model $Y = W + N + WxN + B(WxN) + F + FxW + FxN + FxWxN + FxB(WxN)$, where Y is either spikelet number, flowering time or above ground biomass, W is water, N is nutrient, F is family, and B is block. Block and

family effects were treated as random and block was nested within the water x nutrient term. As there appear to be differences between blocks which represented the same environment, a simpler model was also analyzed. This model was trait = environment + family + environment x family, with environment representing the eight different blocks. Tukey's honestly significant difference method was used to determine which environments and blocks within environments were significantly different from each other in their effects on plant growth. Correlations between fitness and flowering time and between fitness and biomass were analysed using Pearson's correlation coefficient in SPSS statistical software. Pearson's correlations of family means were also carried out across environments to determine the degree of interdependence between the different trait values in the different environments. Families which were significantly different from the parental trait values within each environment were identified using the Dunnett's test. By equating the observed with the expected mean squares, variance components were calculated for the F6 generation for genotype, environment and the genotype X environment interaction term. This was done to determine what proportion of the total variance was attributable to each of these factors.

Results

Significant differences were found between replicates of the same environment using Tukey's honestly significant difference test (Figures 3.2, 3.3, 3.4). This shows, therefore, that despite attempts to hold the environment constant apart from the manipulation of water and nutrients, there are other environmental factors in the greenhouse which can not be controlled and which affect the growth of the plants. However, despite this there are differences between the environmental treatments. Fitness was significantly affected by block effects, family x water x nutrient effects and block x family effects but not by the main treatment effects of water and nutrient (Table 3.1a). Flowering time was significantly affected by all factors except family x water x nutrient (Table 3.2a) and above ground biomass was significantly affected by water, nutrient, water x nutrient and family x water x nutrient (Table 3.3a).When the data was re-analyzed with each of the eight blocks representing a different environment it was found that family, environment and the family x environment effect were significant for all three traits (Tables 3.1b, 3.2b, 3.3b).

Further evidence for the presence of GxE interactions can be found by examining the rank order of the families in the different environments. If there is no change in the rank order then this indicates that there are no GxE interactions. This, however, was not found to be the case. There is a change in rank order of genotypes in all environments for fitness, flowering time and above ground biomass (Figures 3.5, 3.6, 3.7) although flowering time is less affected by the environment than either fitness or above ground biomass. Correlations across environments determined that flowering time was significantly correlated among all environments (Table 3.4) while fitness and above

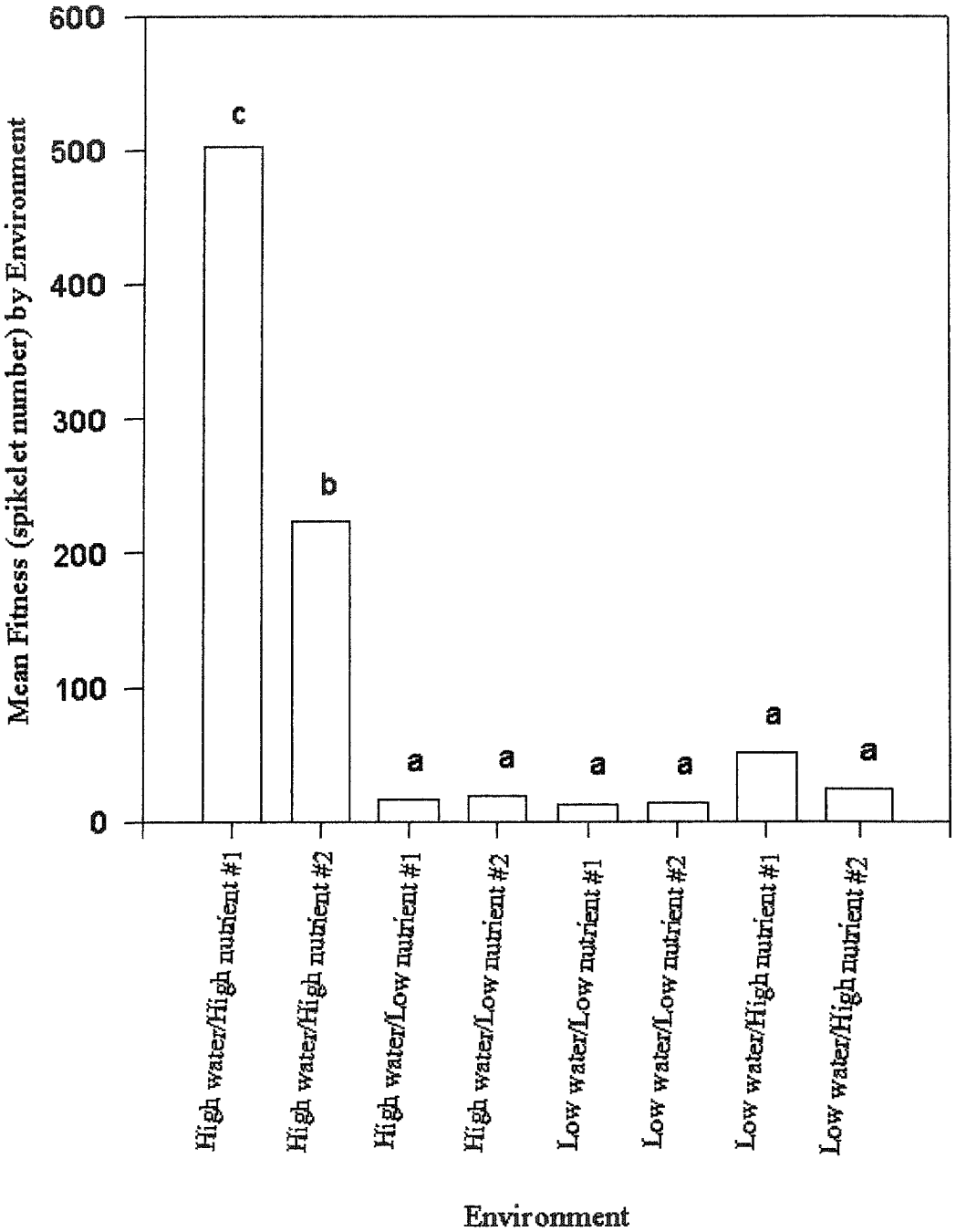

Figure 3.2: Mean fitness (spikelet number) by environment. Tukey's test was used to determine significant differences between environments. Environments belonging to the same homogenous subset are indicated by the letters a, b or c.

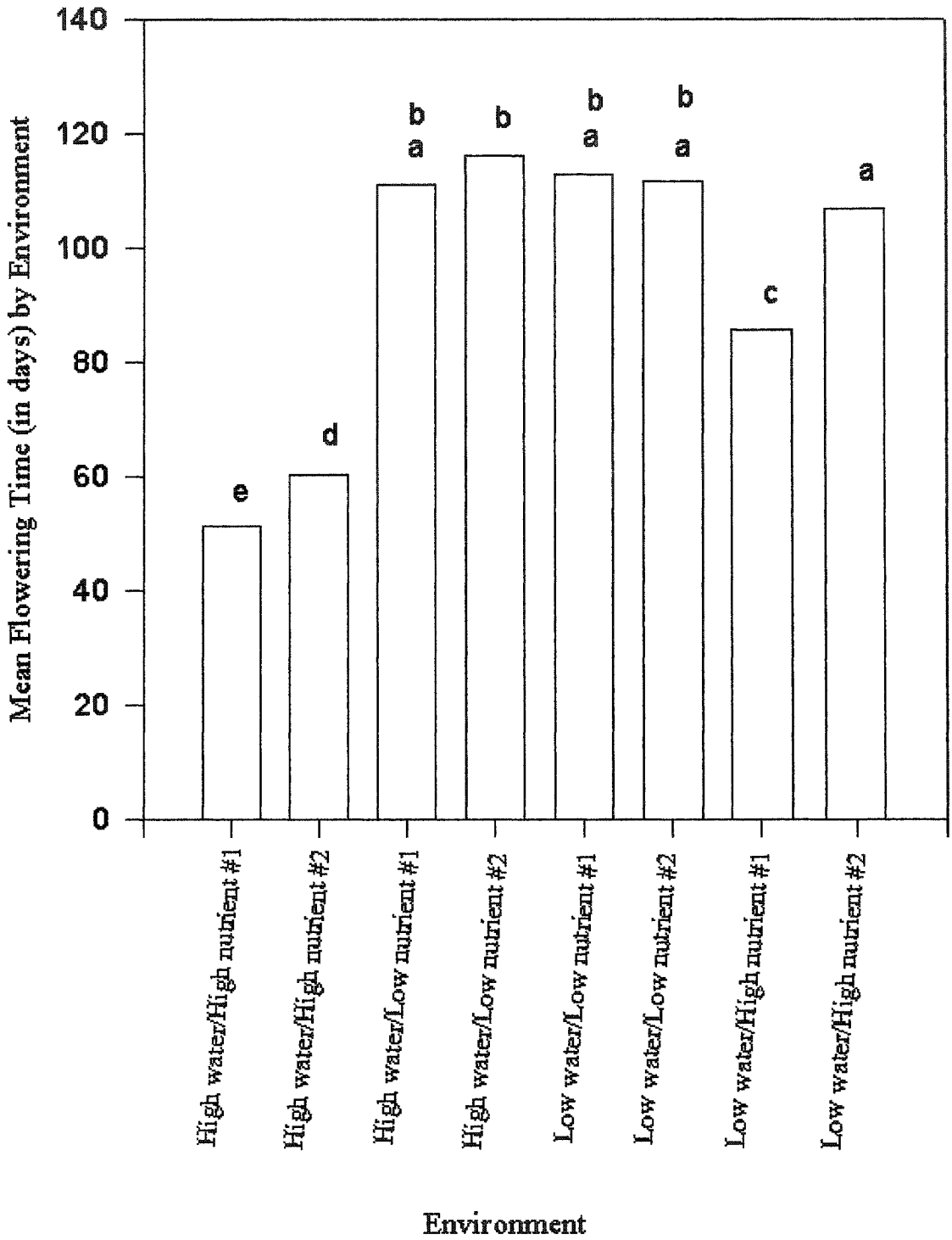

Figure 3.3: Mean flowering time (in days) by environment. Tukey's test was used to determine significant differences between environments. Environments belonging to the same homogenous subset are indicated by the letters a, b, c, d or e.

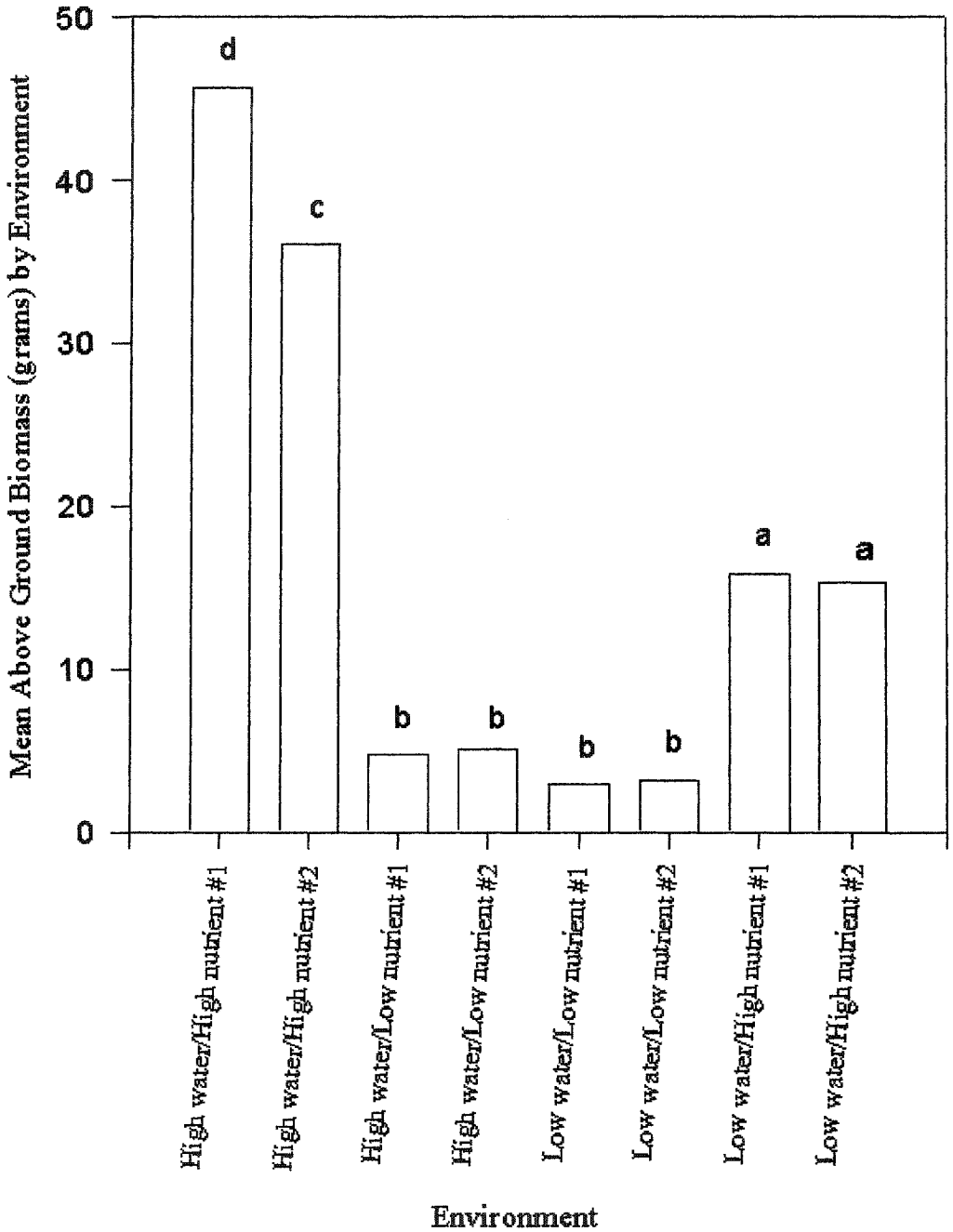

Figure 3.4: Mean above ground biomass (in grams) by environment. Tukey's test was used to determine significant differences between environments. Environments belonging to the same homogenous subset are indicated by the letters a, b, c, or d.

Table 3.1 : Analysis of variance on fitness (spikelet number) of mesic and xeric ecotypes and 12 recombinant families of *A. barbata*. (a) Complete model analyzing each environmental factor separately. (b) Condensed model treating each block as a separate environment.

Source	df	Mean Square	F-value
a) Complete Model			
Water	1	5993457	5.22
Nutrient	1	7432112	6.31
Water X Nutrient	1	5670670	4.94
Block within (water X nutrient)	4	1123234	44.94****
Family	13	122844	1.52
Family X Water	13	50980	1.01
Family X Nutrient	13	80322	1.59
Family X Water X Nutrient	13	50469	2.01*
Block (water X nutrient) X Family	52	25127	2.11****
Error	772	11889.44	
b) Condensed Model			
Environment	7	3436367.23	289.03 ****
Family	13	122843.52	10.33****
Env. X Family	91	41039.82	3.45****
Error	772	11889.44	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 3.2 : Analysis of variance on flowering time of mesic and xeric ecotypes and 12 recombinant families of *A. barbata*. (a) Complete model analyzing each environmental factor separately. (b) Condensed model treating each block as a separate environment.

Source	df	Mean Square	F-value
a) Complete Model			
Water	1	83946	8.49*
Nutrient	1	274793	24.89***
Water X Nutrient	1	92595	13.76*
Block within (water X nutrient)	4	6900.56	9.47****
Family	13	54594	6.74****
Family X Water	13	3751.88	6.62***
Family X Nutrient	13	4916.89	8.68***
Family X Water X Nutrient	13	566.42	0.77
Block (water X nutrient) X Family	52	733.53	1.68**
Error	747	436.41	
b) Condensed Model			
Environment	7	70653.82	161.90****
Family	13	54593.76	125.10****
Env. X Family	91	1716.65	3.93****
Error	747	436.41	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 3.3 : Analysis of variance on above ground biomass of mesic and xeric ecotypes and 12 recombinant families of *A. barbata*. (a) Complete model analyzing each environmental factor separately. (b) Condensed model treating each block as a separate environment.

Source	df	Mean Square	F-value
a) Complete Model			
Water	1	40717	29.43**
Nutrient	1	129288	92.08***
Water X Nutrient	1	30174	22.20 **
Block within (water X nutrient)	4	1296.17	62.20****
Family	13	149.49	0.97
Family X Water	13	108.77	1.29
Family X Nutrient	13	129.68	1.54
Family X Water X Nutrient	13	84.29	4.06****
Block (water X nutrient) X Family	52	20.72	0.60
Error	787	34.57	
b) Condensed Model			
Environment	7	28326.80	800.03****
Family	13	375.60	10.61****
Env. X Family	91	232.40	6.56****
Error	788	35.40	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

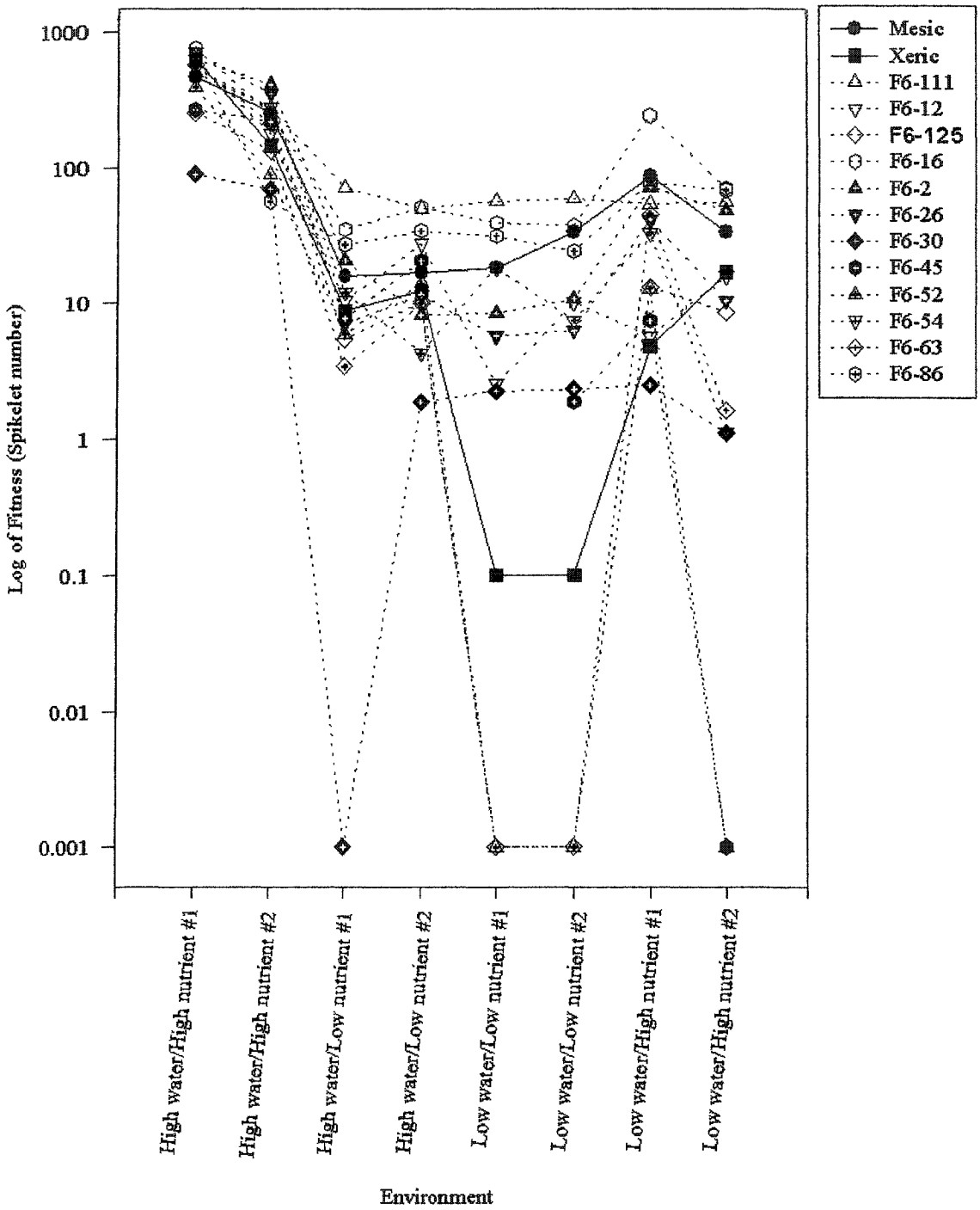

Figure 3.5: Mean fitness (measured as spikelet number) of mesic and xeric ecotypes and 12 F6 families of *A. barbata* grown in eight different environments.

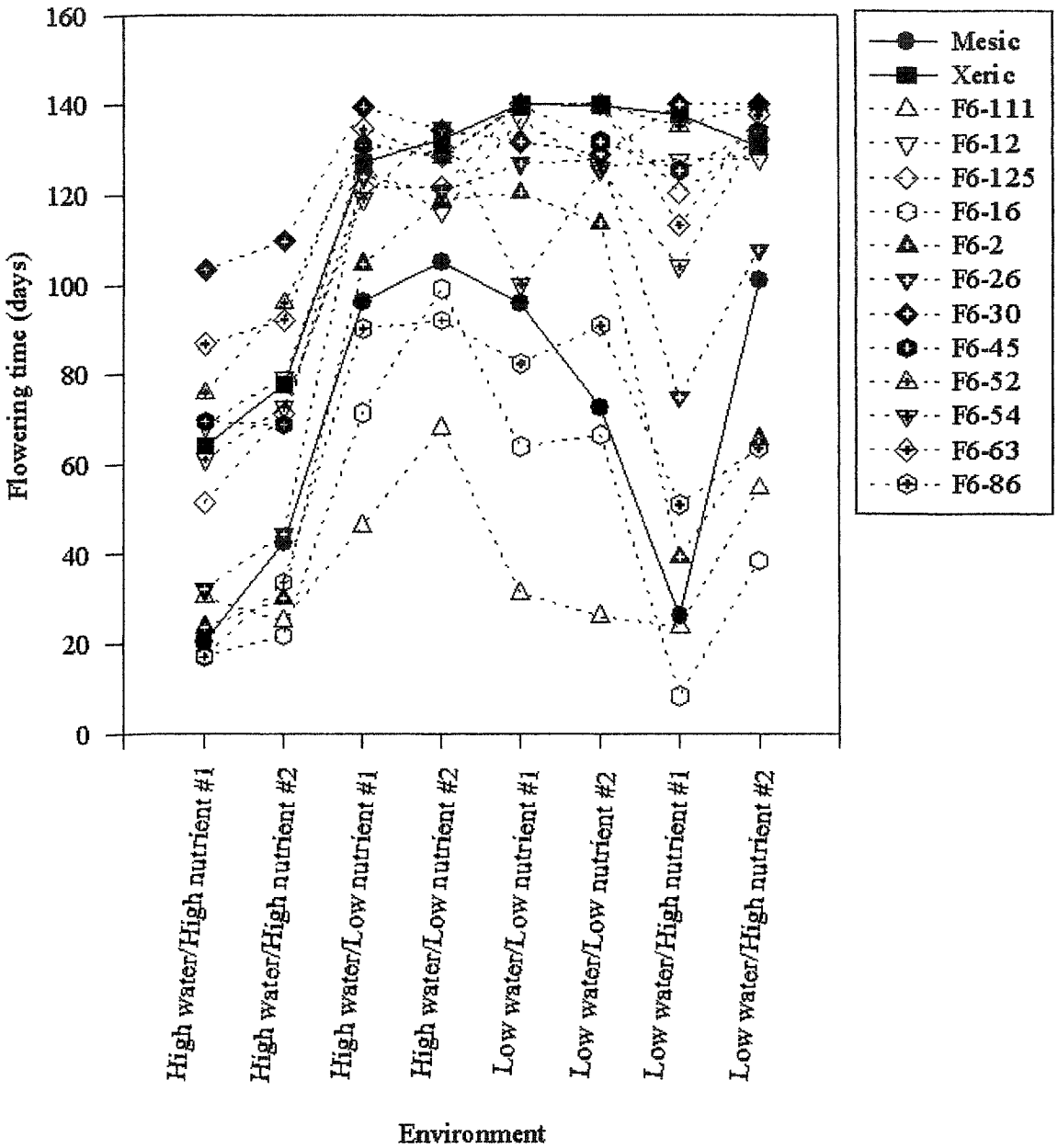

Figure 3.6: Mean flowering time (measured as day first flowering occurred) of mesic and xeric ecotypes and 12 F6 families of *A. barbata* grown in eight different environments.

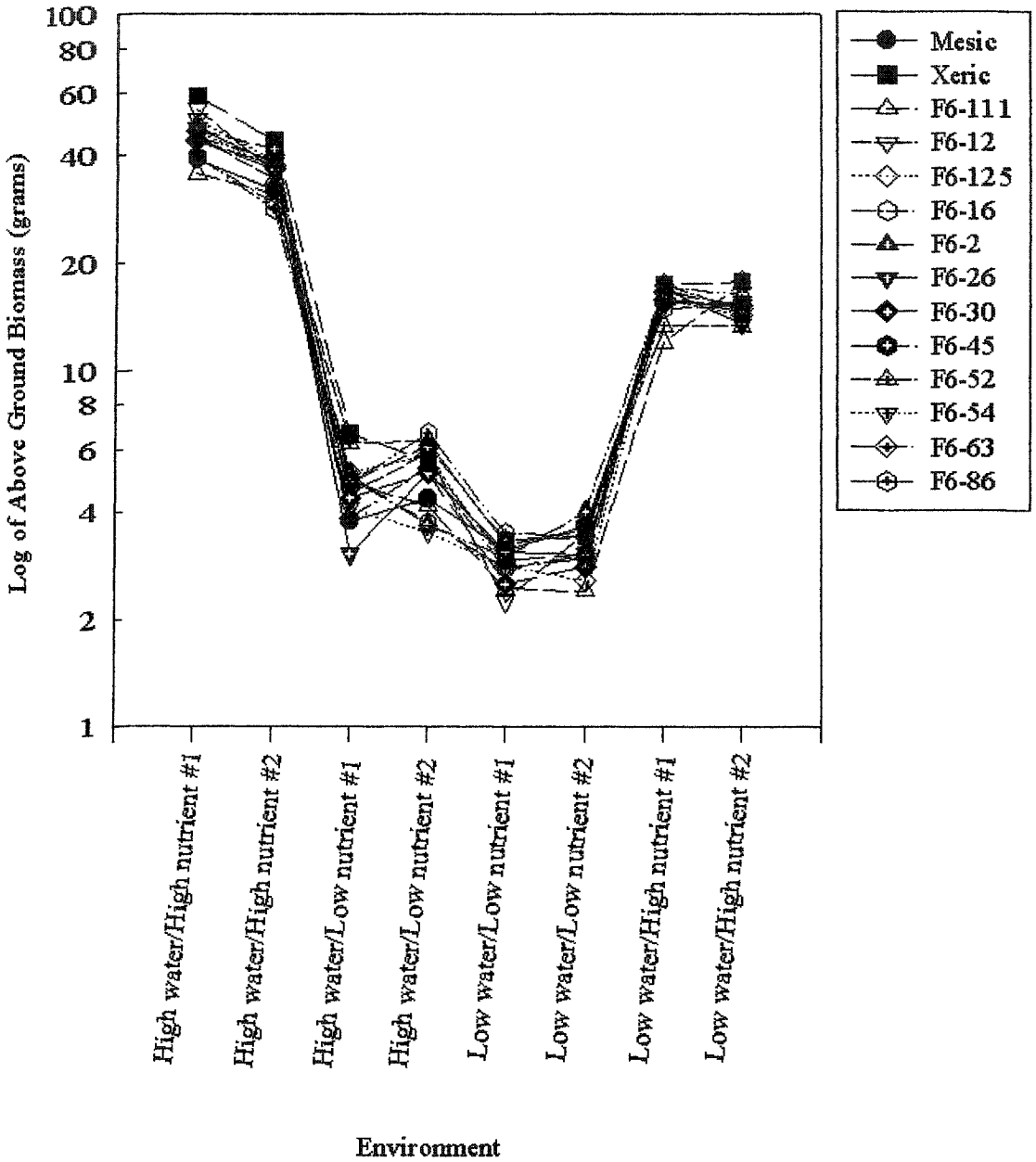

Figure 3.7: Mean above ground biomass (grams) of mesic and xeric ecotypes and 12 F6 families of *A. barbata* grown in eight different environments.

Table 3.4: Correlations of family means (mesic and xeric ecotypes and 12 recombinant F6 families) for flowering time across eight different greenhouse environments.

Correlations								
	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
High Water/ High Nutrient #1		0.960**	0.763**	0.698**	0.654*	0.672**	0.888**	0.844**
High Water/ High Nutrient #2			0.842**	0.764**	0.743**	0.760**	0.925**	0.916**
High Water/Low Nutrient #1				0.934**	0.955**	0.959**	0.871**	0.894**
High Water/Low Nutrient #2					0.874*	0.923**	0.781**	0.820**
Low Water/Low Nutrient #1						0.952**	0.819**	0.819**
Low Water/Low Nutrient #2							0.853**	0.822**
Low Water/High Nutrient #1								0.920**
Low Water/High Nutrient #2								

*P<0.05 **P<0.01

ground biomass had much lower, often non-significant correlations among environments (Tables 3.5, 3.6). As in Chapter 2 fitness and flowering time were found to be significantly correlated (r= -0.908) while fitness and above ground biomass were not (r=0.480).

A Dunnett's test was performed to determine if transgressive segregation was occurring in any of the environments for all three traits. While extreme phenotypes that fall outside the parental range were present in all environments for both fitness and flowering time (Tables 3.7, 3.8), families that were significantly different from the mean parental values were found in only three environments for fitness and seven environments for flowering time. Above ground biomass exhibits less transgressive segregation (Table 3.9) than either fitness or flowering time. Although there are extreme phenotypes present in five of the eight environments, none of the families were significantly different from the parental weights.

Variance components for genotype, environment and GxE were calculated for all three traits (Table 3.10). For both fitness and above ground biomass the majority of the variation was attributable to differences between environment, but the GxE component was considerably larger than the genotype component. In contrast, the majority of the variation for flowering time was attributable to the differences between genotypes, with limited variation due to either environment or GxE.

Table 3.5: Correlations of family means (mesic and xeric ecotypes and 12 recombinant F6 families) for fitness (spikelet number) across eight different greenhouse environments.

Correlations								
	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
High Water/ High Nutrient #1		0.643*	0.340	0.417	0.361	0.314	0.470	0.589*
High Water/ High Nutrient #2			0.407	0.202	0.406	0.379	0.375	0.538*
High Water/Low Nutrient #1				0.818**	0.935**	0.922**	0.463	0.760*
High Water/Low Nutrient #2					0.810**	0.816**	0.622*	0.762**
Low Water/Low Nutrient #1						0.944**	0.617*	0.799**
Low Water/Low Nutrient #2							0.596*	0.788**
Low Water/High Nutrient #1								0.749*
Low Water/High Nutrient #2								

*P<0.05 **P<0.01

Table 3.6: Correlations of family means (mesic and xeric ecotypes and 12 recombinant F6 families) for above ground biomass across eight different greenhouse environments.

Correlations								
	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
High Water/ High Nutrient #1		0.795**	0.289	0.262	-0.110	0.394	0.460	0.064
High Water/ High Nutrient #2			0.180	0.064	-0.001	-0.003	0.486	0.123
High Water/Low Nutrient #1				0.281	0.111	-0.066	-0.127	0.318
High Water/Low Nutrient #2					0.368	0.199	-0.250	-0.322
Low Water/Low Nutrient #1						-0.519	0.075	-0.385
Low Water/Low Nutrient #2							-0.072	0.071
Low Water/High Nutrient #1								-0.097
Low Water/High Nutrient #2								

*P<0.05 **P<0.01

Table 3.7: Rank order of families from highest to lowest fitness in eight different environments. * indicates those families which are significantly different from mean mesic; # indicates those families which are significantly different from mean xeric fitness value.

	Environments							
	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
	F6-16	F6-2*	F6-111	F6-16	F6-111	F6-111	F6-16*	F6-16
	F6-26	F6-26	F6-16	F6-111	F6-16	F6-16	**Mesic**	F6-86
	F6-86	F6-86	F6-86	F6-86	F6-86	**Mesic**	F6-86	F6-111
	F6-2	F6-54	F6-2	F6-12	F6-54	F6-86	F6-2	F6-2
	Xeric	F6-111	**Mesic**	F6-45	**Mesic**	F6-2	F6-111	**Mesic**
Family	F6-12	**Mesic**	F6-54	**Mesic**	F6-2	F6-12	F6-63	**Xeric**
	F6-125	F6-16	F6-12	F6-52	F6-26	F6-54	F6-26	F6-12
	F6-54	F6-45	**Xeric**	**Xeric**	F6-12	F6-26	F6-54	F6-26
	F6-111	F6-125	F6-45	F6-26	F6-30	F6-30	F6-125	F6-125
	Mesic	F6-12	F6-26	F6-125	F6-125	F6-45	F6-52	F6-63
	F6-52	**Xeric**	F6-52	F6-63	F6-45	**Xeric**	F6-45	F6-54
	F6-45*	F6-63	F6-125	F6-2	F6-52	F6-125	F6-12	F6-30
	F6-63*	F6-52	F6-63	F6-54	F6-63	F6-52	**Xeric**	F6-45
	F6-30*	F6-30	F6-30	F6-30	**Xeric**	F6-63	F6-30	F6-52

Table 3.8: Rank order of families from earliest to latest flowering time in eight different environments. * indicates those families which are significantly different from mean mesic; # indicates those families which are significantly different from mean xeric fitness value.

	Environment							
	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
	F6-86	F6-16	F6-111*	F6-111*	F6-111*	F6-111*	F6-16	F6-16*
	F6-16	F6-111	F6-16	F6-86	F6-86*	F6-16	F6-111	F6-111*
	Mesic	F6-2	F6-86	F6-16	F6-16*	**Mesic**	**Mesic**	F6-86*
Family	F6-2	F6-86	**Mesic**	**Mesic**	**Mesic**	F6-86	F6-2	F6-2*
	F6-111	**Mesic**	F6-2	F6-12	F6-54	F6-2	F6-86	**Mesic**
	F6-26	F6-26	F6-54	F6-2	F6-2	F6-54	F6-26	F6-26
	F6-125	F6-45	F6-125	F6-26	F6-26	F6-12	F6-54	F6-12
	F6-54	F6-125	F6-26	F6-125	F6-30	F6-26	F6-63	**Xeric**
	Xeric	F6-54	F6-12	F6-45	F6-12	F6-30	F6-125	F6-125
	F6-12	**Xeric**	**Xeric**	F6-52	F6-125	F6-45	F6-45	F6-54
	F6-45	F6-12	F6-45	F6-63	F6-45	**Xeric**	F6-12	F6-45
	F6-52	F6-63	F6-52	**Xeric**	F6-52	F6-125	F6-52	F6-63
	F6-63	F6-52	F6-63	F6-30	F6-63	F6-52	**Xeric**	F6-30
	F6-30#	F6-30#	F6-30	F6-54	**Xeric**	F6-63	F6-30	F6-52

Table 3.9: Rank order of families from highest to lowest above ground biomass in eight different environments. * indicates those families which are significantly different from mean mesic; # indicates those families which are significantly different from mean xeric fitness value.

	Environment							
Family	High Water/ High Nutrient #1	High Water/ High Nutrient #2	High Water/ Low Nutrient #1	High Water/ Low Nutrient #2	Low Water/ Low Nutrient #1	Low Water/ Low Nutrient #2	Low Water/ High Nutrient #1	Low Water/ High Nutrient #2
	Xeric	**Xeric**	**Xeric**	F6-86	F6-86	F6-2	F6-16	F6-111
	F6-12	F6-45	F6-52	F6-52	F6-45	**Mesic**	**Xeric**	**Xeric**
	F6-54	F6-2	F6-125	F6-45	F6-52	F6-52	F6-2	F6-2
	F6-26	F6-54	F6-2	F6-12	**Mesic**	F6-12	F6-26	F6-12
	F6-45	F6-26	F6-16	F6-125	F6-26	F6-86	F6-30	**Mesic**
	F6-125	F6-63	F6-111	**Xeric**	F6-2	F6-45	F6-54	F6-86
	F6-52	F6-125	F6-45	F6-63	**Xeric**	F6-26	F6-45	F6-54
	F6-2	F6-30	F6-86	F6-26	F6-125	**Xeric**	F6-125	F6-30
	F6-30	F6-12	F6-12	F6-30	F6-54	F6-16	F6-63	F6-63
	F6-63	F6-52	F6-30	**Mesic**	F6-63	F6-63	**Mesic**	F6-16
	F6-86	**Mesic**	F6-54	F6-111	F6-16	F6-54	F6-12	F6-45
	F6-16	F6-111	F6-63	F6-16	F6-30	F6-30	F6-86	F6-125
	Mesic	F6-16	**Mesic**	F6-2	F6-111	F6-125	F6-52	F6-26
	F6-111	F6-86	F6-26	F6-54	F6-12	F6-111	F6-111	F6-52

Table 3.10: Variance components and proportion of total variance for F6 generation attributable to genotype, environment and genotype X environment interaction for fitness, flowering time and above ground biomass.

Variance	Fitness-variance component	Fitness proportion of total variance	Flowering Time-variance component	Flowering Time-proportion of total variance	Above Ground Biomass-variance component	Above Ground Biomass-proportion of total variance
Genotype	778.17	0.05	428	0.54	0.26	0.002
Environment	14336.15	0.83	282.15	0.36	124.87	0.99
Genotype X Environment	2144.49	0.12	75.74	0.10	0.677	0.005

Discussion

The main purpose of this experiment was to examine the later generation hybrids to determine if there were families that could out perform the parental ecotypes in novel environments. Numerous studies have documented that transgressive segregation occurs frequently in plant populations and most likely provides the material for rapid adaptation to novel environments (reviewed in Rieseberg *et al.* 1999; Rieseberg *et al.* 2003). However, the production of extreme phenotypes in parental environments will not necessarily result in offspring that do well in other environments. In order for the hybrid offspring to be capable of colonizing novel habitats, the transgressive phenotype must contribute to adaptation in the novel habitat. Transgressive phenotypes were found in all environments for fitness, flowering time, and above ground biomass. These extreme phenotypes could lead to niche differentiation between certain hybrid genotypes and the parental ecotypes. As in Chapter 2, not all of the extreme phenotypes are significantly different from the parental value with entire families, rather than simply a few individuals, falling outside the parental range. The fact that some families perform better than the parental ecotypes in all novel environments indicates that these families may be capable of colonizing a non-parental environment and possibly providing the material for adaptive evolution.

The relative fitness of the hybrid may depend on the environmental conditions under which it is grown. If hybrid fitness is not independent of the environment this will be seen as GxE interactions. While the main effects of water and nutrients themselves do not significantly affect fitness, there was a significant effect of block as well as all of the interaction terms in the full model ANOVA and for the environment x family effect in

the simple ANOVA (Table 3.1). These results indicate that there are GxE interactions occurring for fitness, although it seems that fitness responds not just to the water/nutrient regime which was imposed, but also to some other unidentified factor. Although the majority of the total variation in fitness is attributable to differences among environments, indicating that fitness is sensitive to changes in the environment, the fact that the variation that is attributable to the GxE interaction is larger than the variation attributable to the genotype alone lends support to the finding that different families do respond differently to a change in environment. Two families were found to have consistently high fitness, with F6-111 having the highest fitness in three of the eight environments and F6-16 having the highest fitness in four of the eight environments and the second highest fitness in three other environments. Interestingly, in the two high water/high nutrient environments the F6-111 family ranked fifth (out of fourteen families) in one environment and ninth in the other and in the two low water/ high nutrient environments it ranked third and fifth. This may indicate that the F6-111 family performs better in environments with low nutrient levels. Family F6-30 had the poorest fitness in five of the environments, with its best performance (ninth) in both the low water/low nutrient environments. Again, indicating that this genotype may do better under harsher conditions. The change in rank order of genotypes (Figure 3.5) together with the results from the ANOVA indicates that with the exception of family F6-16 the fitness of the different genotypes changes depending on the environment in which the plant is grown. This may allow selection to act on different genotypes in the different environments, and to eventually obtain close to the optimum phenotype for a particular environment, in turn leading to local adaptation for that particular environment. Family

F6-16 however, appears to have a high fitness regardless of the environment in which it is grown. This is consistent with the formation of a genotype which is broadly adapted to all the greenhouse environments. This in turn may allow family F6-16 to colonize new areas regardless of the environmental conditions without experiencing the lower fitness which would be experienced by those families which respond differently to the different environments .

Although initially the experiment was designed to distinguish between the effects of water and nutrients, the results of the Tukey's tests indicate that there were differences between what were intended to be replicates of the same environments. The main differences observed were between the two high water/ high nutrient environments. These two environments were placed in different rooms in the greenhouse so it seems likely that attempts to hold all factors, except the water and fertilizer regime, constant was not successful. For this reason, while it seems that families are responding to a particular level of water or fertilizer, it is difficult to determine with certainty if this is all the plants are responding to. Nonetheless, it is clear that there are GxE interactions taking place for fitness, and that there are certain genotypes that, if exposed to a novel environment, may be able to outperform the parental ecotype.

Above ground biomass was significantly affected by the interaction of family x water x nutrient as well as water x nutrient but not for block x family. It was, however, significantly affected by the interaction of environment x family (Table 3.3) indicating the presence of GxE interactions. There was also a substantial change in the rank order of the families between the environments (Figure 3.7), which again is consistent with there being GxE interactions for this trait. As was expected those plants grown in an

environment which received adequate water and nutrients had a greater biomass than those grown in the harsher environments. Variation among environments accounted for 99% of the total variation, which is consistent with above ground biomass being sensitive to the environment in which it is grown. This result was consistent with that of other studies which examined growth rate and nutrient availability (Elberse *et al.* 2003; Verhoeven *et al.* 2004). Similar to the results of Chapter 2, there was no correlation between fitness and above ground biomass (r=0.480). However, as with the results of Chapter 2, these results may change when the plants are grown under natural conditions where they have to compete for resources.

Flowering time, however, appears to be less sensitive to changes in the environment than either fitness or above ground biomass, with only 36% of the total variation being attributable to environmental differences. Flowering time was not significantly affected by family x water x nutrient but varied significantly among both block x family and environment x family (Table 3.2). Although the rank order of the families did change slightly between environments (Figure 3.6), indicating there are GxE interactions, the order of the families did not change as much as that of both fitness and above ground biomass. The proportion of total variance which is attributable to family, environment and the interaction between the two is consistent with flowering time being less sensitive to changes in the environment than either fitness or above ground biomass. Just over half of the total variation is attributable to the main effect of genotype. Those families that flowered early in one environment tended to flower early in all the environments. Overall these results are interesting, given the correlation between fitness and flowering time (r=-0.803). One would expect that if there is little change in the rank

order of the families across environments for flowering time then fitness would behave in a similar manner. The success of an individual in an unpredictable environment is going to depend on the ability of the individual to respond appropriately to the conditions (Quinn and Wetherington 2002). However, in this experiment it seems that a family's success does not necessarily depend on when flowering occurred. For instance, although family F6-111 flowered consistently early, it did not have the highest fitness in the high water/high nutrient environments. This seems to indicate that some factor apart from flowering time is affecting the number of spikelets produced. It may be that although some families had an earlier flowering time, other families were able to produce spikelets more rapidly resulting in a greater number of spikelets. Other adaptive strategies such as putting energy into producing larger seeds in an attempt to increase the chance of the offspring's success may also have come into play. However, as seed size was not recorded during this experiment, which of these scenarios is most likely remains undetermined.

Genetic correlations between trait values expressed in different environments can be used to determine the degree of independence between the trait values in the different environments (Via 1994). If there is a significant correlation between trait values, then the values are not independent; therefore, there may be a genetic constraint on the evolution of the trait value towards a more adaptive value for that particular environment (Via 1994). Correlations for fitness show that while some environments are less correlated with others, still others are significantly correlated with all of the environments (Table 3.5). The non-zero correlations indicate that although there is a genetic constraint on the evolution of a more adaptive reaction norm a new reaction norm may still evolve

even though none of the trait values are completely independent of each other. As the response to the various environments will differ if some genotypes are better adapted to one environment than the other, translating into smaller genetic correlations between environments (Bell 1992), this indicates some genotypes must be better adapted to certain environments than others. The highest correlations for fitness tend to be between replicates of the same environments which is consistent with the alleles responsible for this trait affecting fitness in a similar manner in these environments. This may indicate that the plants react in a manner more similar to each other in the replicate environments than they do in the other environments. Above ground biomass shows very little significant correlation among environments (Table 3.6) which would facilitate the evolution towards a more adaptive reaction norm (Via 1994). Flowering time, however, shows significant correlation between all environments (Table 3.4), which means there is little independent genetic variation between the environments (Via 1994). This may occur when the alleles responsible for the trait, in this case flowering time, affect the trait value in the same manner in all environments (Via 1994). This result corresponds with those of the variance calculation results in that the majority of the total variance for flowering time is due to differences between families rather than the response of the plant to the environment.

Overall this experiment demonstrated that GxE interactions do exist for the later generation *A. barbata* hybrids and that, while some families are more fit than the parental ecotypes under certain environmental conditions, other hybrid families may be unfit under the same conditions. This is consistent with other studies (Campbell and Waser 2001; Johnston *et al.* 2001b), which also documented that hybrid offspring are not

uniformly unfit relative to their parents but rather, that fitness depends on the environment in which the hybrid is grown. The combination of transgressive segregation in all environments with the presence of GxE interactions shows that hybridization of *A. barbata* can result in offspring which are capable of outperforming the parental ecotypes and of colonizing novel habitats. This in turn could lead to range expansion, local adaptation and possible speciation. However, this experiment was performed under greenhouse conditions with little competition between plants. Therefore, while the results indicate that hybridization does not always lead to unfit offspring in *A. barbata*, whether the same results would be found if the plants were exposed to novel habitats, where they have to compete for resources, is not known.

Chapter 4

Fitness of early and late generation hybrids of *Avena barbata* in mesic and xeric environments

Introduction

The fitness of both the early and the late generation *A. barbata* hybrids have previously been tested in a greenhouse environment (Chapter 2). I determined that both dominance and epistatic gene effects are involved in the fitness of the hybrid offspring. Transgressive phenotypes were also found in this experiment with entire families falling outside the parental range, indicating that transgressive segregation is a genetic rather than an environmental phenomenon. This indicates that there may be families that are capable of outperforming the parental ecotypes and possibly leading to range expansion and adaptation to a novel environment. Also, the fitness of the late generation hybrids was tested to determine if any hybrid families were capable of outperforming the parental ecotypes in novel environments as well as examining whether GxE interactions exist. This experiment determined that not only are there families that do outperform the parents in novel environments, but that GxE interactions do occur, which means that the individual hybrid families responded differently to the different environments. The families which perform well in a particular environment may be adapted to that environment, which again could contribute to range expansion of this species.

However, these two experiments were performed in the greenhouse and did not answer a number of questions. Still unanswered are: 1) whether the putative co-adapted gene complexes in the parental ecotypes contribute to local adaptation, 2) whether there are later generation hybrids which can outperform the parents in the parental environment

as opposed to a novel environment and 3) whether the dominance and epistatic effects which were seen in the greenhouse (Chapter 2) have the same effect on fitness in the parental environments, as a trait which is measured in different environments may be influenced at least in part by different genes (Falconer 1990).

Local adaptation is a common phenomenon in plant species that occurs when a phenotype is adapted to local environmental conditions (Kingsolver *et al.* 2002; Perez de la Vega 1996). The hybridization of two individuals which are adapted to different environments may result in offspring with a lower fitness than either parent in the parental environments (Waser and Price 1994). This can be seen when *Artemisia tridentata* ssp. *tridentata* hybridizes with *A. tridentata* ssp. *vaseyana*. These subspecies hybridize in a narrow region between the two parental environments. Studies have demonstrated that the hybrids outperform the parents within the hybrid zone and the parent subspecies outperform hybrids and the other parental subspecies in each of their natural environments (Wang *et al.* 1997). Reciprocal transplants of *Gilia capitata* ssp. *capitata* and *G. capitata* ssp. *chamissonis* have demonstrated that there is local adaptation for several traits, with the native subspecies performing better than the immigrant seed in both sites (Nagy and Rice 1997). In *Iris brevicaulis* and *I. fulva*, the *I. brevicaulis*-like hybrids and *I. fulva*-like hybrids are more likely to be found in habitat similar to the habitat of the closest parental species (Johnston *et al.* 2001b). *Ipomopsis aggregata* has also been found to be locally adapted as seed set was found to be higher with crossing distances of 1-10 m than from either the selfed or 100 m outcross pollinations (Waser and Price 1989). Also, fitness of the offspring was found to decline with distance from maternal parent indicating the presence of local adaptation (Waser and Price 1989). This

illustrates that although heterosis may result in an increase in fitness in some instances (i.e. high seed set), the effect of local adaptation can counteract any benefits gained through heterosis.

A. barbata appears to have co-adapted gene complexes which are thought to confer adaptation to two climatic zones, one of which is a warm, arid climate and the other a cooler moister climate (Clegg and Allard 1972). The frequencies of each ecotype tend to be correlated with the environment to such an extent that, as the size of each zone changes each year, the frequency of each ecotype also changes (Perez de la Vega *et al.* 1991). Also, other ecotypes, each with their own multi-locus allelic combinations have been found in patches which have unusual environmental conditions (Perez de la Vega *et al.* 1991). Reciprocal transplant experiments between parents and hybrid offspring of several species have demonstrated that not only can the parents be locally adapted to their habitats (Schluter 1995; Hatfield and Schluter 1999), but it is also possible for the hybrid offspring to be locally adapted to their particular habitat regardless of whether the habitat is simply intermediate to the two parental environments or novel to that of the parents (Emms and Arnold 1997; Wang *et al.* 1997).

A reciprocal transplant experiment suggested that there is weak selection which results in the mesic and xeric ecotypes being selected for in their respective environments (Jain and Rai 1980). Another study demonstrated that there are quantitative characters which differ between the mesic and xeric ecotypes and which have a genetic component (Hamrick and Allard 1975). The mesic ecotype tends to be shorter, with more tillers, and both flowers and matures its seeds earlier than the xeric ecotype (Hamrick and Allard 1975). If these co-adapted gene complexes in *A. barbata* are in fact locally adapted to

specific environments, then the hybrid offspring should have a lower fitness in each of the parental environments, and it should be less likely that transgressive phenotypes, that out-perform the parental ecotypes in the parental environments, will occur. If, however, these multi-locus allelic combinations are not co-adapted gene complexes which are adapted to the local conditions, then the production of hybrids will not necessarily result in offspring with lower fitness than the parents in the parental habitat. Instead, this may result in transgressive phenotypes which will allow for some hybrid offspring to outperform the parental ecotypes. Also, as traits measured in different environments are partly influenced by different genes (Falconer 1990), it is possible that the dominance and epistatic gene effects, which were seen in Chapter 2, may not be as pronounced in the parental environments. While the use of early generation hybrids will not determine with certainty whether there are co-adapted gene complexes adapted to the local environment, they will help to determine whether there are dominance or epistatic gene effects contributing to fitness in the parental environments. The use of the later, more stabilized hybrid generation will help to determine if local adaptation is occurring since, even if there are beneficial dominance effects that counteract hybrid breakdown in the early generation hybrids, by the F6 generation the hybrids are 97% homozygous and therefore any beneficial dominance effects will no longer be present.

Methods

Two sites in California were used in this experiment: the Sierra Foothills Research and Extension Centre (SFREC), which is considered a xeric site, and the Hopland Research and Extension Centre (HREC), which is considered a mesic site (Hutchinson 1982). The native *A. barbata* found at SFREC were mainly xeric, with some patches of mesic. The total annual rainfall for the year 2002 was 627.13 mm and for the year 2003 was 784.35 mm. However, the rainfall amounts were not spaced evenly throughout the years. Instead, December 2002 and April 2003 were very wet with the other months being relatively dry. The vegetation in this area was less dense then that found at HREC. HREC was densely populated by plants appearing to be of the mesic ecotype. The total annual rainfall at HREC for the year 2002 was 967.74 mm and for the year 2003 was 964.7 mm, with December 2002 and April 2003 again being very wet while the other months were relatively dry.

The same twenty-five F6 families which were used in Chapter 2 were used in this experiment. A total of 50 mesic and 50 xeric seeds, 100 F2 seeds (50 from family F2-A and 10 from each of family F2-B,C,D,E and F), and 400 F6 seeds (16 seeds from each of the 25 families) were planted at each site in November 2002. Three seeds from the remaining 163 F6 families were also planted for use in a QTL mapping study (Gardner in prep), however, they are not included in any of the analysis in this study. The seeds were germinated by placing them in petri dishes lined with wet filter paper and placing them in the refrigerator for three days. At this point they were removed and placed in the dark at room temperature to germinate. Once the seeds had germinated they were planted in Ray Leach cone-tainers (Stuewe and Sons Inc. Oregon), in Sunshine Mix #3 (Sungro,

Vancouver British Columbia), a commercial seedling germination mixture containing peat moss, vermiculite, gypsum, and dolomitic lime , and allowed to become established for approximately one week in a greenhouse at HREC. After the seedlings were established they were planted at the two sites. Each site was divided into three blocks, with the plants divided equally between the blocks, and the position of the plants within each block was randomized. The sites were fenced off in an attempt to keep any herbivores out. Holes were punched in the ground using a dibble, a large metal rod with a pointed end, and the seedlings were planted by placing the cone-tainers, with the bottoms cut out, in the holes. Removing the bottoms allowed the roots of the plant to grow into the native soil. Plants were located on a grid with approximately 30 cm between plants and the adjacent natural vegetation was left undisturbed. The plants were allowed to grow until senescence had been reached in early June 2003 at which point fitness, quantified as the number of spikelets produced, was recorded. Above ground biomass was measured by cutting the plants off at soil level, placing them in paper bags, drying them in a drying oven for four days at 50 °C and obtaining their dry weights. As it was not possible for someone to be in California for the entire course of this experiment flowering time was not recorded.

This experiment is analysed and discussed as two separate experiments. As in Chapters 2 and 3 plants which did not survive were coded as missing values, while those plants which had not produced spikelets were assigned a fitness of zero. Although mortality at SFREC was low (12 of the 479 plants did not survive) the situation at HREC was quite different. One hundred and fifty-one of the 528 plants did not survive at this site. However, as there was no difference in survivorship between families and as again

the mortality seems to be random with some plants putting up culms prior to mortality (i.e. mortality may be due to grazing rather than due to selection against a particular genotype) these individuals were coded as missing data rather than as exhibiting a fitness of zero. The first analysis examines the results to determine the long term vs. short term consequences of hybridization, as in Chapter 2. This allows for a discussion of whether dominance and epistatic effects contribute to fitness in the natural habitat. Each environment is analysed separately and the statistical analysis of this section is the same as that of Chapter 2. The second analysis examines the results to determine whether GxE interactions occur in the parental environments and, therefore, whether local adaptation is occurring. The statistical analyses of this section is the same as that of Chapter 3 with the following exceptions. Instead of twelve F6 families as in Chapter 3, the same twenty-five families used in Chapter 2 are used in this study. The model used for the ANOVA is trait = environment + block (within environment) + family + family x environment + error, where trait is either spikelet number or above ground biomass. The family means for each trait are plotted across the two environments to determine if there is a change in the magnitude or direction of the reaction norm.

Results

Long term vs. short term field experiment

None of the interaction terms was significant at either site and are therefore not included in the models presented. Analysis of variance shows that there were significant generation effects for fitness (Table 4.1) and significant generation and family effects for above ground biomass (Table 4.2) at HREC. At SFREC significant effects were found for generation and family for both fitness and above ground biomass (Table 4.3, 4.4). Linear contrasts showed significant differences between the F6 vs. parents and F6 vs. F2 for both fitness and above ground biomass in both environments.

At HREC dominance and epistatic gene effects for fitness were much larger than the additive gene effect, with the dominance gene effect being the largest, while for above ground biomass the dominance gene effect was again the largest, with the additive gene effect being larger than the epistatic gene effect (Table 4.5). Both dominance and epistatic effects were substantially larger than the additive gene effect for fitness and above ground biomass at SFREC (Table 4.6).

Family means for both fitness and above ground biomass were found to be significantly correlated at both HREC ($r=0.809$ $p<0.0001$) (Figure 4.1) and SFREC ($r=0.935$, $p<0.0001$) (Figure 4.2).

Using a Dunnett's test I found that at HREC only one family was significantly different from the mid-parent for fitness, family F6-145, which did significantly worse than the mid-parent (Figure 4.3). For above ground biomass families F6-105, F6-145, F6-26 and F6-6 all weighed significantly less than the mid-parent (Figure 4.4). At SFREC two

Table 4.1: Analysis of variance on fitness (spikelet number) to evaluate effects of long term and short term hybridization in *Avena barbata* at HREC. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	40.87	1.13
Generation	2	306.93	10.45**
Family within generation	30	32.22	0.89
Error	352	36.32	
Linear Contrasts			
Parents vs. F2	1	45.04	1.24
F2 vs. F6	1	148.53	4.09*
Parents vs. F6	1	549.2	15.11***
Error	354	36.35	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 4.2: Analysis of variance on above ground biomass to evaluate effects of long term and short term hybridization in *Avena barbata* at HREC. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	0.10	1.34
Generation	2	1.01	4.39*
Family within generation	30	0.17	2.36***
Error	355	0.07	
Linear Contrasts			
Parents vs. F2	1	0.01	0.26
F2. vs. F6	1	1.21	17.27****
Parents vs. F6	1	1.21	17.35****
Error	357	0.07	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 4.3: Analysis of variance on fitness (spikelet number) to evaluate effects of long term and short term hybridization in *Avena barbata* at SFREC. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	255.94	0.96
Generation	2	4378.74	5.79*
Family within generation	30	524.98	1.97**
Error	476	265.96	
Linear Contrasts			
Parents vs. F2	1	530.18	1.99
F2 vs. F6	1	6800.43	25.57****
Parents vs. F6	1	3961.38	14.9***
Error	478	265.92	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 4.4: Analysis of variance on above ground biomass to evaluate effects of long term and short term hybridization in *Avena barbata* at SFREC. Linear contrasts were used to determine presence of heterosis and epistasis.

Source	df	Mean Square	F value
Block	2	1.02	1.11
Generation	2	20.66	7.00**
Family within generation	30	1.10	2.16****
Error	476	0.93	
Linear Contrasts			
Parents vs. F2	1	2.15	2.32
F2 vs. F6	1	31.37	33.85****
Parents vs. F6	1	19.42	20.95****
Error	478	0.93	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 4.5: Ecotype and generation means ± SE. Additive, dominance, and epistatic effects of fitness and above ground biomass at HREC calculated using expectations for generation means as found in Mather and Jinks (1982). 95% confidence limits for differences between two means were calculated according to Sokal and Rohlf (1995).

	Fitness	Above Ground Biomass
Mesic Mean	10.98 ± 0.94	0.77 ± 0.06
Xeric Mean	10.21 ± 2.76	0.40 ± 0.03
F2 Mean	10.45 ± 0.79	0.66 ± 0.05
F6 Mean	7.81 ± 0.25	0.44 ± 0.01
Additive Effect	0.38 ± 5.79	0.18 ± 0.15
Dominance Effect	5.62 ± 1.61	0.47 ± 0.07
Epistatic Effect	2.95 ± 2.36	0.16 ±0.05

Table 4.6: Ecotype and generation means ± SE. Additive, dominance, and epistatic effects of fitness and above ground biomass at SFREC calculated using expectations for generation means as found in Mather and Jinks (1982). 95% confidence limits for differences between two means were calculated according to Sokal and Rohlf (1995).

	Fitness	Above Ground Biomass
Mesic Mean	26.06 ± 2.28	1.72 ± 0.16
Xeric Mean	24.33 ± 2.96	1.09 ± 0.14
F2 Mean	27.82 ± 2.4	1.5 ± 0.16
F6 Mean	18.09 ± 0.79	0.91 ± 0.04
Additive Effect	0.86 ± 7.44	0.32 ± 0.43
Dominance Effect	20.74 ± 4.96	1.3 ± 0.23
Epistatic Effect	7.75 ± 2.13	0.5 ± 0.13

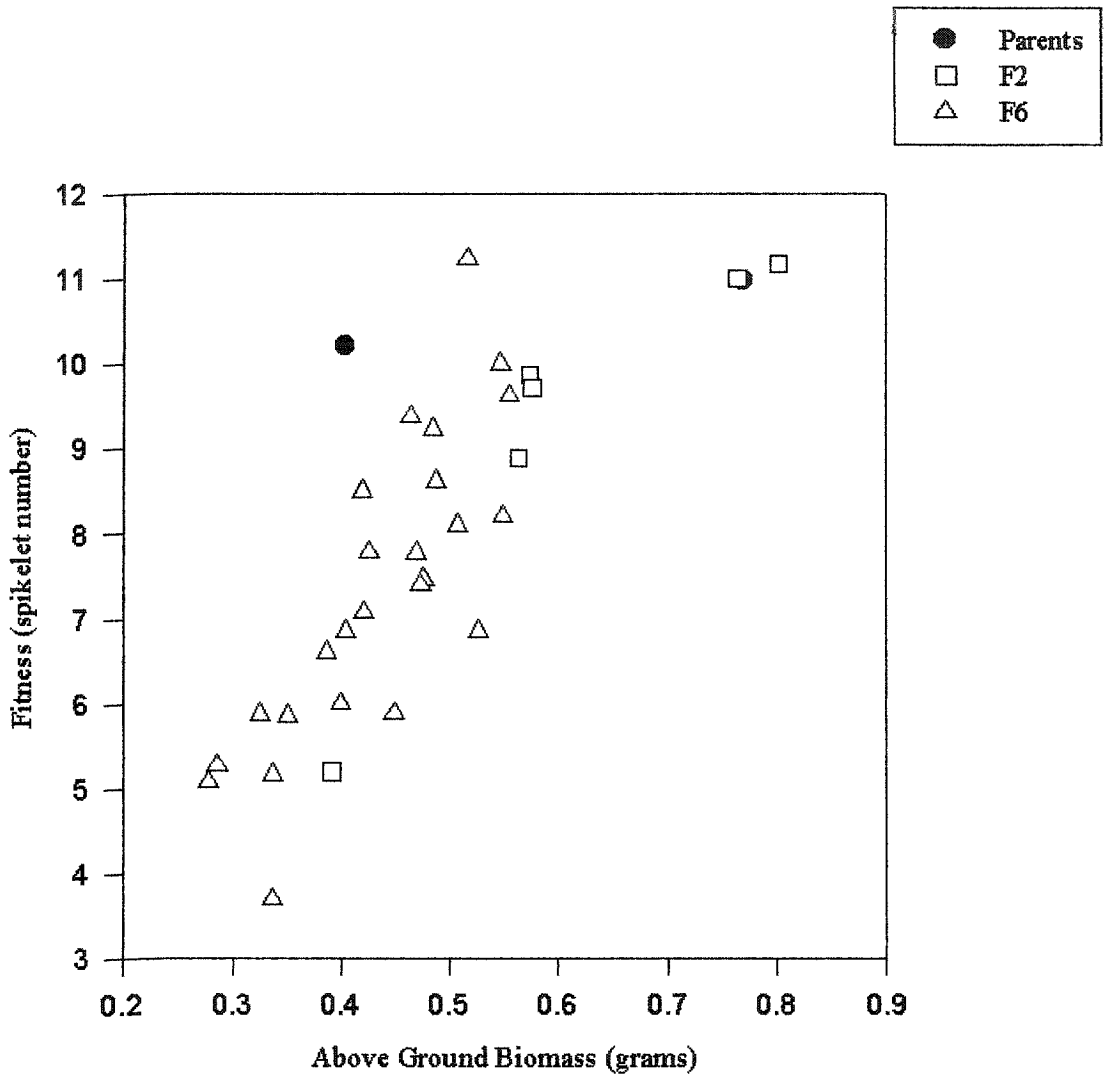

Figure 4.1: Mean fitness of each family vs. mean above ground biomass of each family for mesic and xeric ecotypes, six F2 families and 25 F6 families of *A barbata* grown at HREC. Correlation coefficient= 0.809.

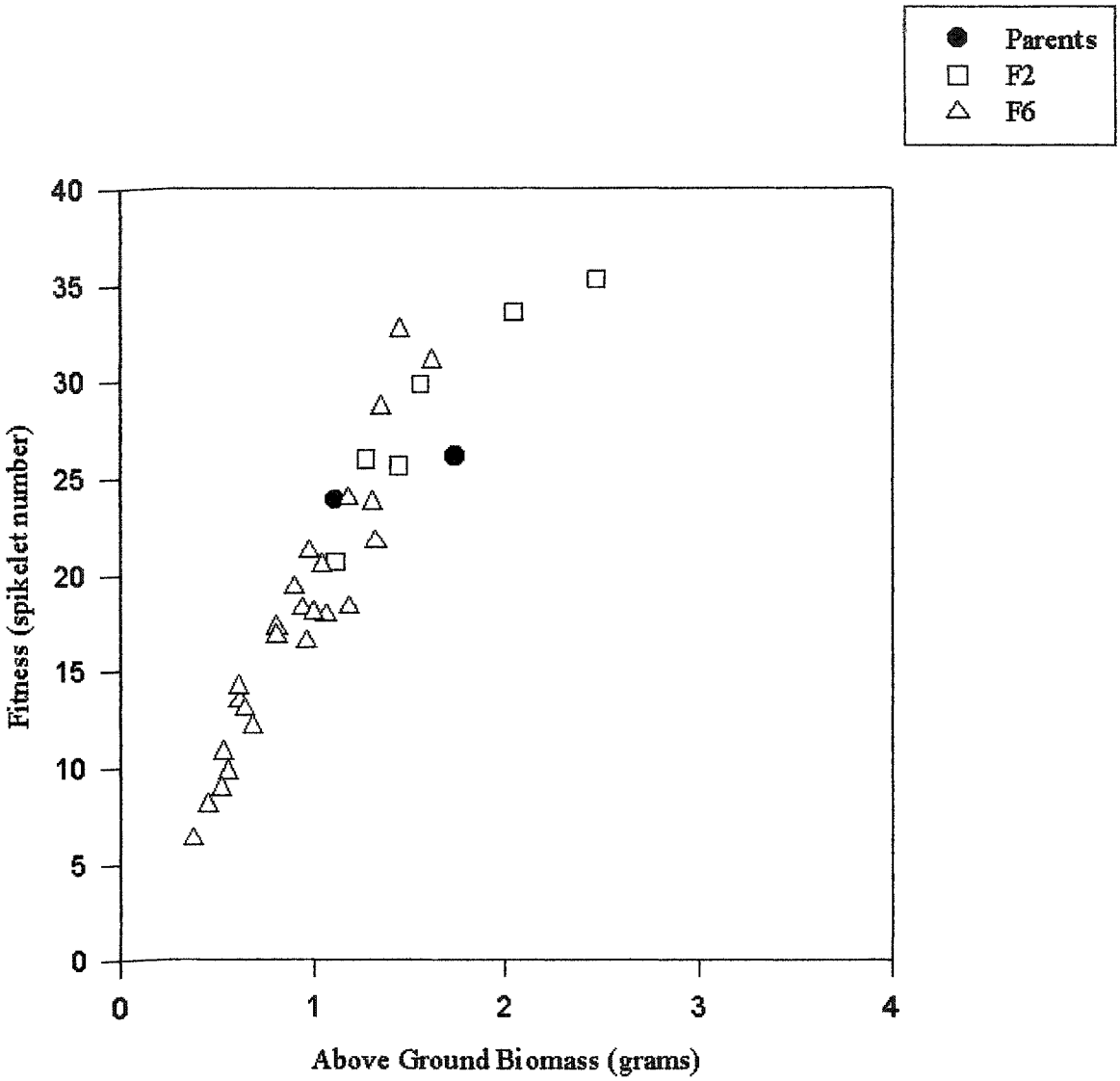

Figure 4.2: Mean fitness of each family vs. mean above ground biomass of each family for mesic and xeric ecotypes, six F2 families and 25 F6 families of *A barbata* grown at SFREC. Correlation coefficient= 0.935.

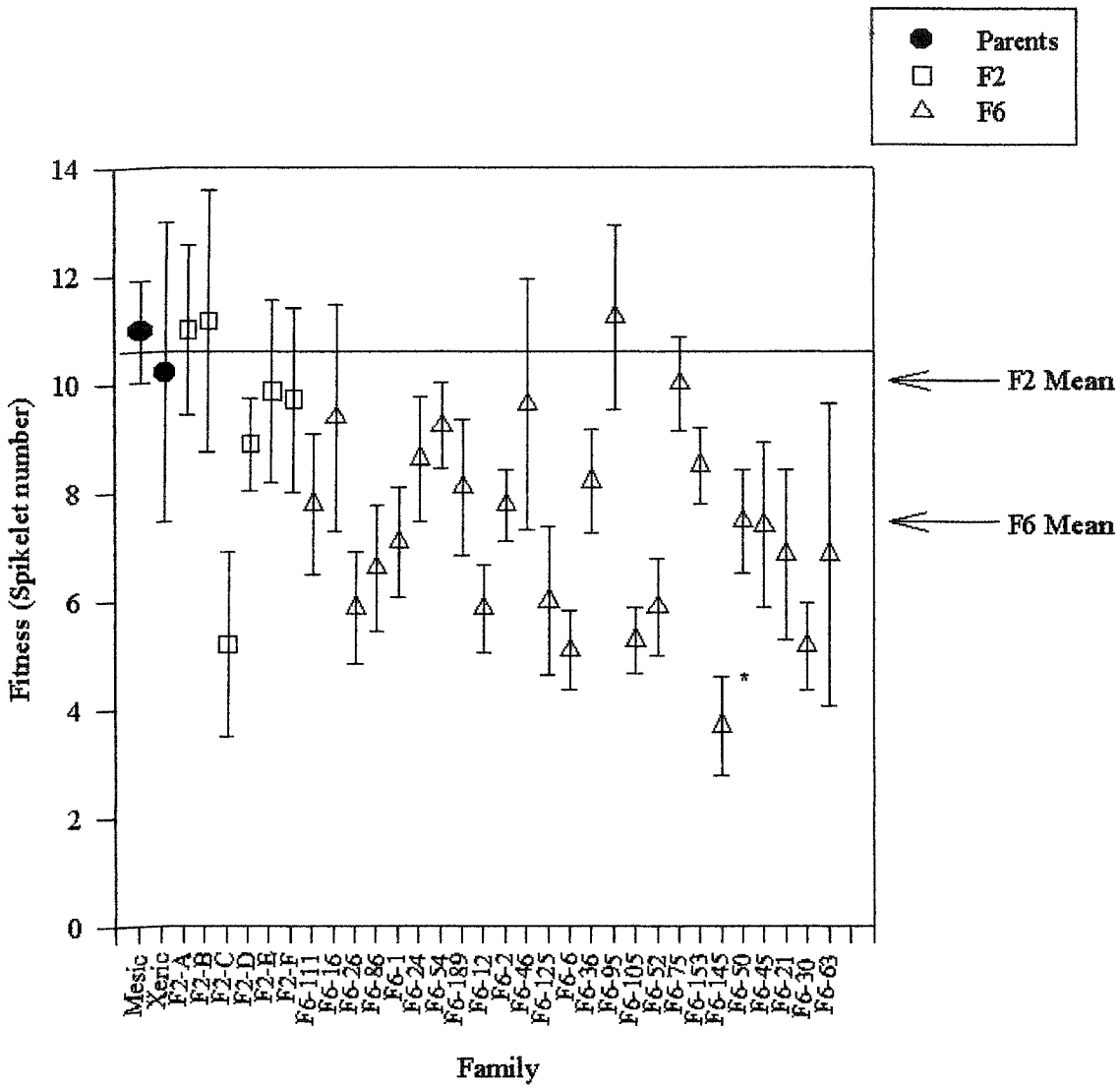

Figure 4.3: Mean fitness (spikelet number) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families at HREC. The families are arranged in the same order as in Chapter 2.

*=significantly different from mid-parent value.

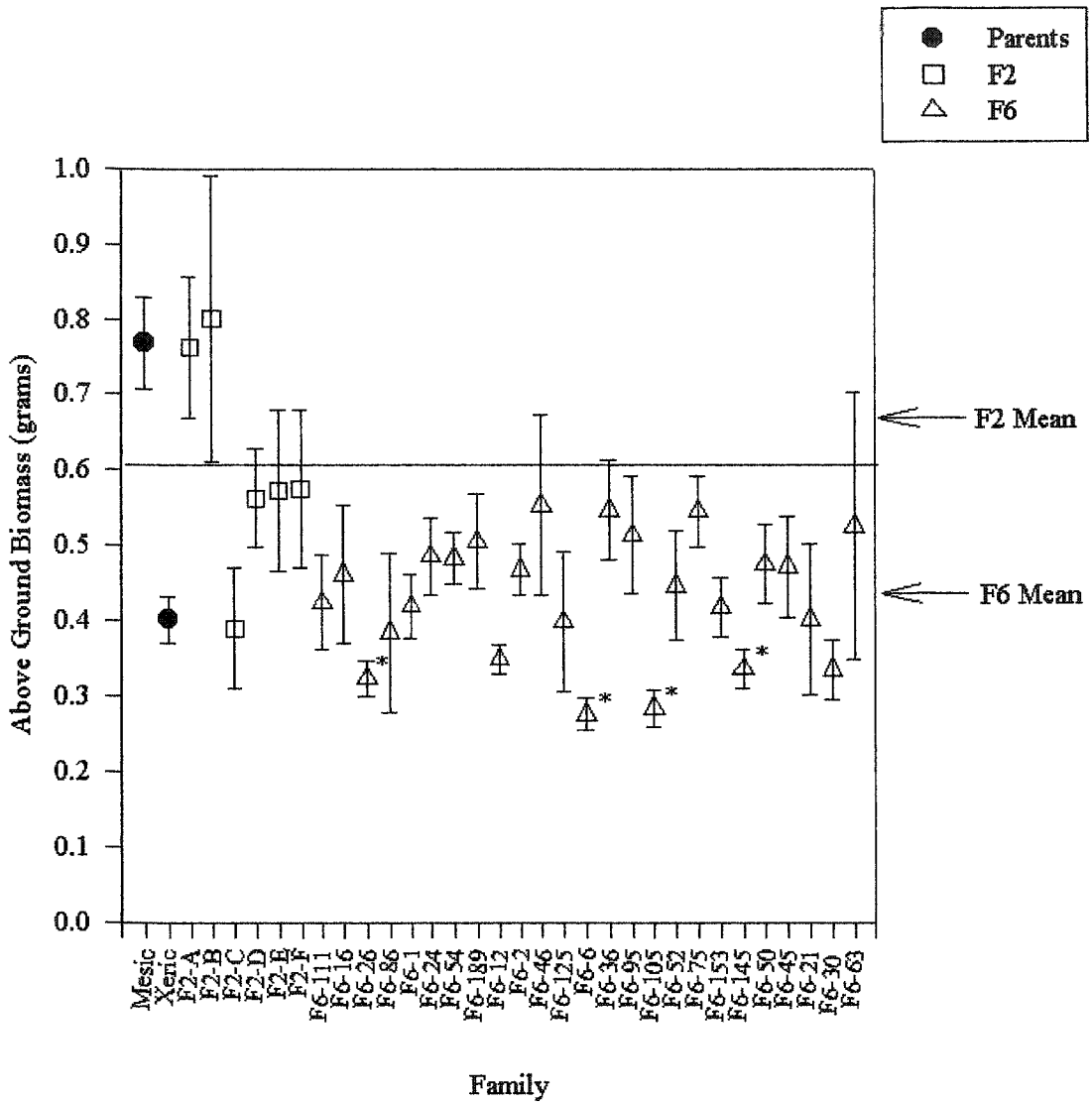

Figure 4.4: Mean above ground biomass (grams) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families at HREC. The families are arranged in the same order as in Chapter 2.

*=significantly different from mid-parent value.

families (F6-145 and F6-105) had significantly lower fitness (Figure 4.5) while one family (F2-B) weighed significantly more and four families (F6-145, F6-105, F6-6 and F6-95) weighed significantly less than the mid-parent value (Figure 4.6).

The Dunnett's test was also used to determine if any of the hybrid offspring families had extreme performances relative to the parental values. Family F6-145 had a fitness significantly lower than the xeric parent at both sites. However, although the differences were not significant, at both sites many families fell outside the parental range with far more families doing worse than the least fit ecotype than those that did better than the most fit ecotype (Figures 4.3, 4.5).

Fitness of F6 generation in parental environments

Both fitness and above ground biomass were greater at SFREC than at HREC. Significant differences were found for the main effects of family and environment for both traits (Tables 4.7, 4.8). However, only above ground biomass showed significant GxE interactions.

Correlations of family means between environments for fitness ($r=0.551$, $p<0.01$) and biomass ($r=0.581$, $p<0.01$) show that the variation in phenotypic response to the environment among genotypes does not seem to be as great in the parental environments as in the novel greenhouse environments (Chapter 3). The rank order of families does not change greatly between the two environments for either fitness or above ground biomass (Figures 4.7, 4.8). The mesic ecotype outperformed the xeric ecotype at both sites. The variance results are similar to those found in Chapter 3 in that the major portion of total variance for both fitness and above ground biomass is attributable to the

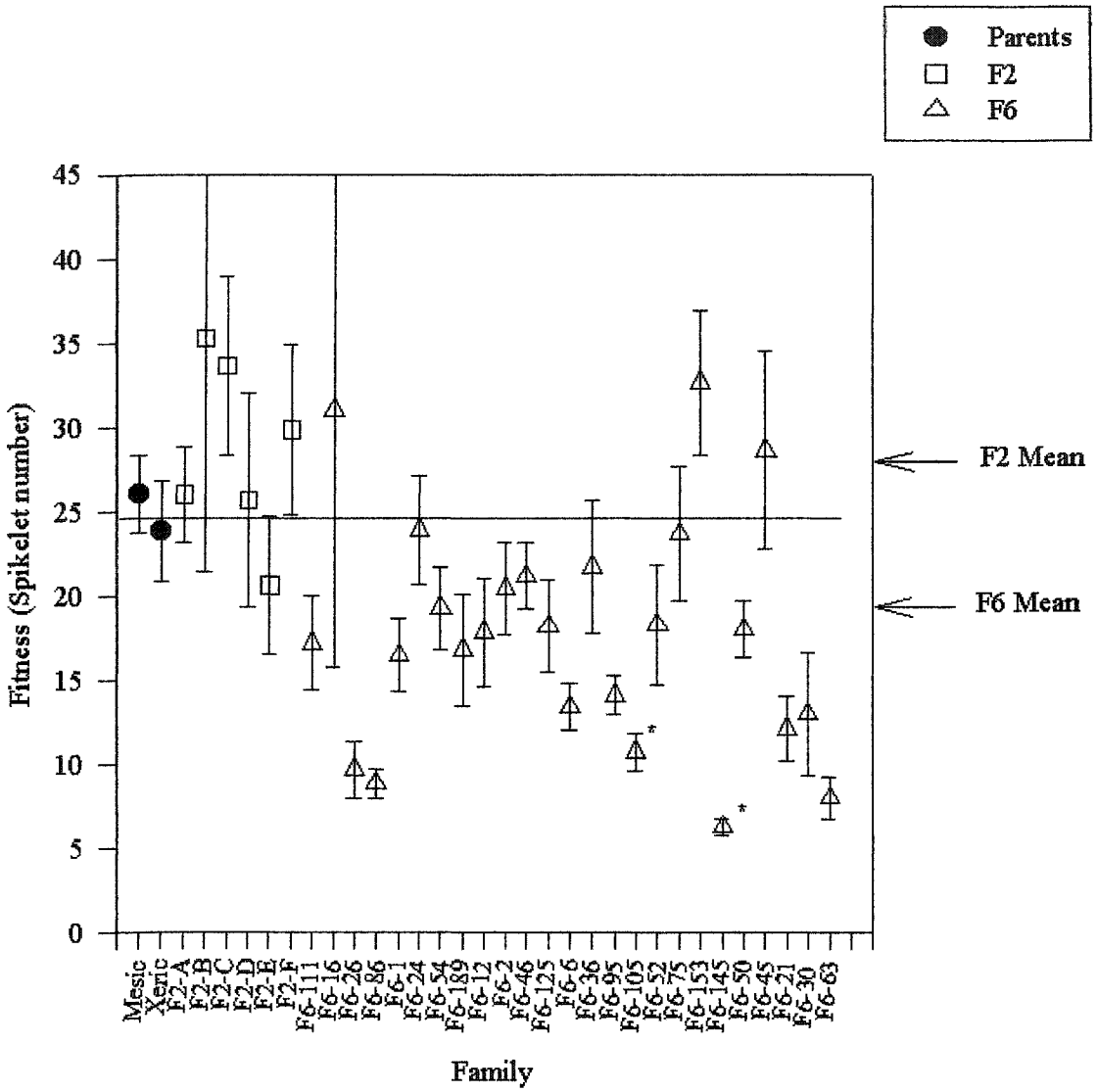

Figure 4.5: Mean fitness (spikelet number) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families at SFREC. The families are arranged in the same order as in Chapter 2.

*=significantly different from mid-parent value.

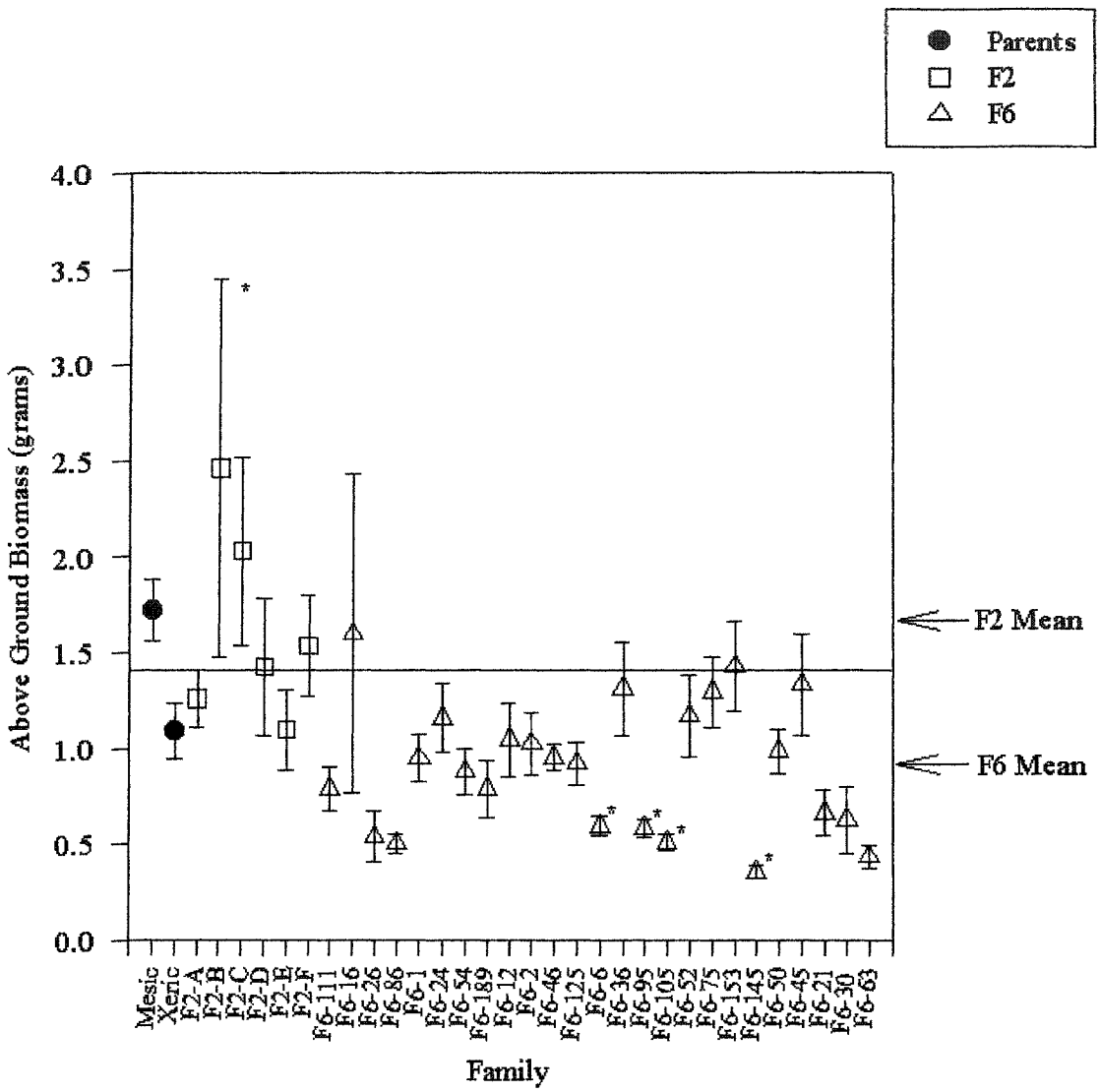

Figure 4.6: Mean above ground biomass (grams) ± standard error of mesic and xeric ecotypes, six F2 families and 25 F6 families at SFREC. The families are arranged in the same order as in Chapter 2.

*=significantly different from mid-parent value.

Table 4.7: Analysis of variance on fitness for *A. barbata* grown in the parental environments in California.

Source	df	Mean Square	F-value
Environment	1	16607.77	117.28****
Block within (Environment)	4	79.96	0.68
Family	26	450.78	2.25*
Environment x Family	26	200.58	1.43
Error	695	139.71	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

Table 4.8: Analysis of variance on above ground biomass for *A. barbata* grown in the parental environments in California.

Source	df	Mean Square	F-value
Environment	1	35.75	83.23****
Block within (Environment)	4	0.19	0.46
Family	26	1.74	2.67**
Environment x Family	26	0.65	1.60*
Error	696	0.38	

*P< 0.05 ** P< 0.01 ***P< 0.001 ****P<0.0001

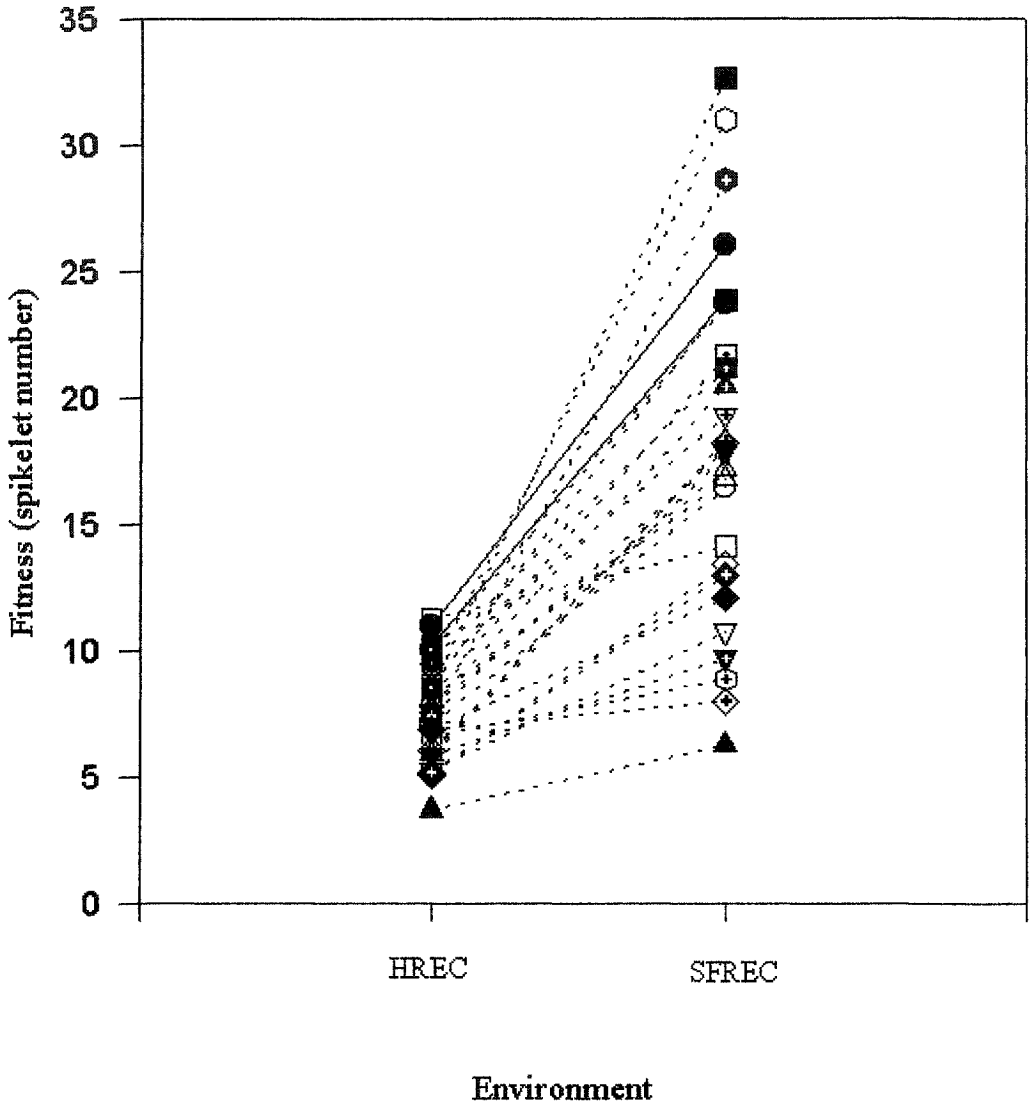

Figure 4.7: Family means for fitness at HREC and SFREC in California. Parental ecotypes are indicated by solid lines, F6 hybrid offspring families by dotted lines. ($r=0.551$, $p<0.01$)

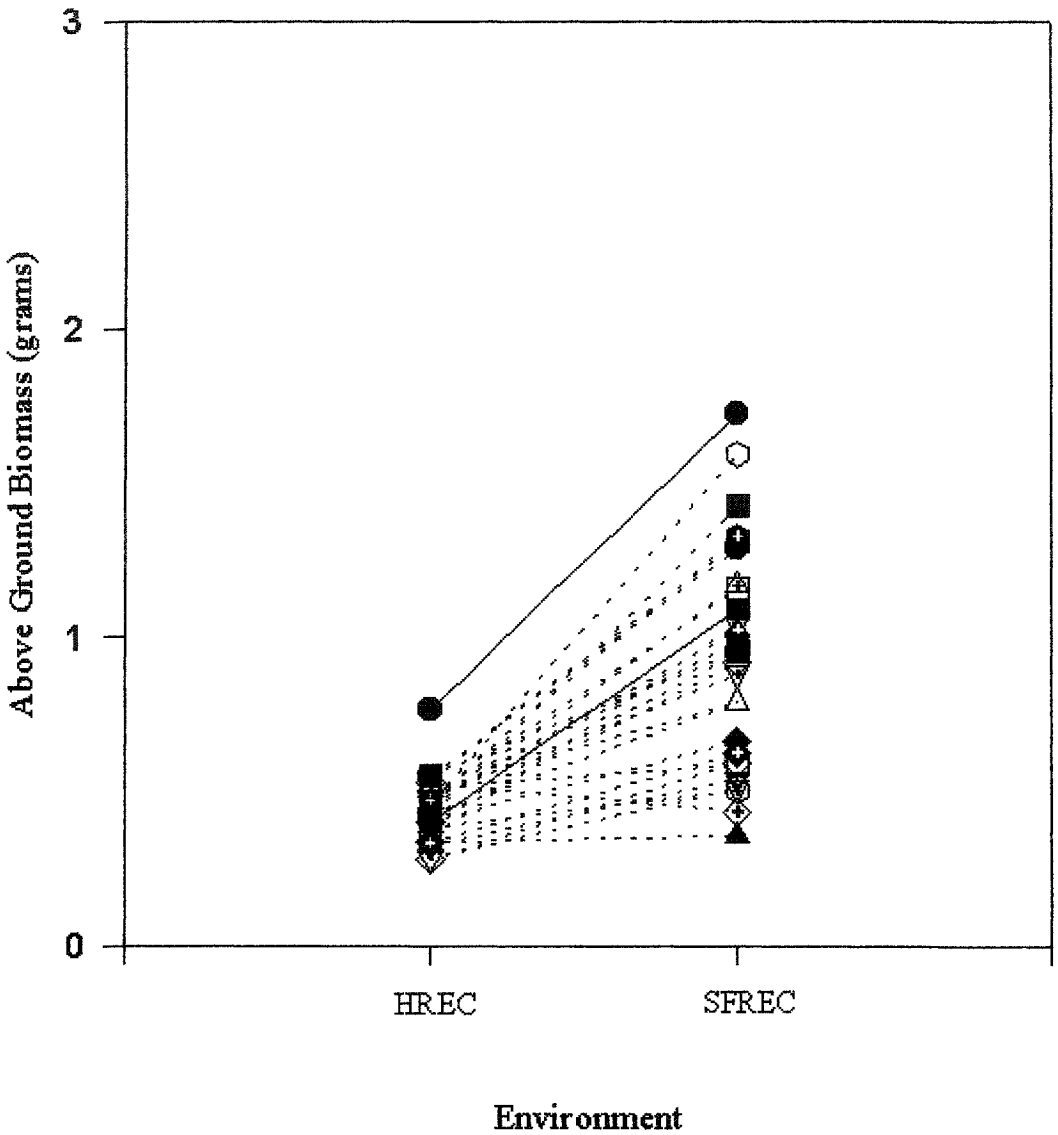

Figure 4.8: Family means for above ground biomass at HREC and SFREC in California. Parental ecotypes are indicated by solid lines, F6 hybrid offspring families by dotted lines. ($r=0.581$. $p<0.01$)

differences between environments. Limited variation was attributable to either genotype or the genotype x environment interaction term (Table 4.9).

Table 4.9: Variance components and proportion of total variance for F6 generation attributable to genotype, environment and genotype x environment interaction for fitness and above ground biomass.

	Fitness-variance component	Fitness-proportion of total variance	Above Ground Biomass-variance component	Above Ground Biomass-proportion of total variance
Genotype	4.93	0.111	0.01	0.108
Environment	33.82	0.759	0.07	0.752
Genotype x Environment	5.79	0.129	0.013	0.139

Discussion

The genetic basis of hybrid fitness in the parental environments was found to be the same as that of the greenhouse study, in that dominance and epistatic gene effects are playing a major role in hybrid fitness. While the parental ecotypes do appear to have co-adapted gene complexes which affect fitness, it is still not clear whether these co-adapted gene complexes lead to local adaptation as there were families of later generation hybrids which were capable of outperforming the parental ecotypes in their own environments. The mesic ecotype outperformed the xeric ecotype at the xeric site; if these ecotypes are locally adapted to their respective environments then it would be expected that each ecotype should exhibit the highest fitness in its own environment.

Long term vs. short term field experiment

The presence of significant generation effects as well as the results of the linear contrasts at both HREC and SFREC indicate that similar to the greenhouse results, there appears to be hybrid vigour in the second generation hybrids which is counteracted by hybrid breakdown. A significant generation effect indicates that the genetic basis of hybrid fitness is not simple additivity. If the underlying genetic basis of the hybrid fitness is simple additivity, there should be no differences found between the F6 generation and either the F2 or the parental generation (Table 1.1). As seen in Chapter 2, the deviation, therefore, of the F6 generation from the parental mean can be taken as evidence for epistatic interactions and although it may seem that the lack of difference between the F2 generation and the parental generation indicates that there is no hybrid vigour or hybrid breakdown present in the early generation hybrids, this is not the case. The deviation of the F6 from the F2 mean can be taken as evidence for the presence of dominance. Again,

there appears to be both a large dominance gene effect and an epistatic gene effect at both sites. *A. barbata* has undergone generations of selection in the field environment whereas the greenhouse is an environment in which there has been no previous selection on these genotypes. If the lower fitness expressed by hybrid offspring was found only in the field environment, then it is possible that this lower fitness could be the result of exogenous selection. However, as the lower fitness is found both in an environment in which *A. barbata* has evolved as well as in an environment in which there is no history of selection, this is consistent with the hypothesis that there are co-adapted gene complexes which contribute to fitness in the parental ecotypes. Therefore, the lower fitness is to some extent the result of endogenous selection. When a trait is measured in different environments, it may be affected at least partially by the same genes (Falconer 1990). The lower fitness of the hybrid offspring which is found in both the greenhouse and the parental environments is consistent with fitness being influenced at least partly by the same genes.

The additive gene effects which are found in the parental environments are much smaller than those found in the greenhouse environment. This is consistent with the theory that selection will reduce the amount of additive genetic variance present (Hartl and Clark 1997). The small additive gene effects which are found in the parental environments, where *A. barbata* has undergone generations of selection, is much less than that which is found in the greenhouse environment, where there has been no history of selection (Chapter 2).

For above ground biomass the main effects of generation and family were found to be significant at both sites (Tables 4.2, 4.4) These results are supported both by the

linear contrasts which show that there are differences between the F6 generation and both the F2 and the parental generation, and by the calculation of the net effect of additive, dominance or epistatic gene effects. This indicates that there are in fact large dominance gene effects as well as additive and epistatic effects at both sites. The results indicate that unlike the greenhouse environment where above ground biomass and fitness do not seem to have the same genetic basis, above ground biomass in a field environment seems to have the same genetic basis as fitness, in that there is hybrid vigour which is counteracted by the break-up of co-adapted gene complexes.

The results from the Dunnett's test indicate that in the parental environments there are no recombinant families that do significantly better in terms of fitness than the mid-parent value. As the epistatic effect for fitness is larger in the field than it was in the greenhouse this may mean that the break-up of co-adapted gene complexes has a larger effect on fitness in the field then in the greenhouse. There is one family in each of the environments which had a significantly higher biomass than the mid-parent; however, overall the recombinant genotypes did much worse than the mid-parent value in the field environments than they did in the greenhouse environments.

Contrary to the results found in both Chapters 2 and 3, there appears to be a positive correlation between fitness and above ground biomass in a natural environment. It seems that if a plant has a larger above ground biomass, it also has a higher fitness (Figures 4.1, 4.2). Since the plants were not confined to a limited area in the field, as they were in the greenhouse, it is possible that they were able to extend their roots and thus access more resources. This result is consistent with bigger plants being able to better

utilize the available resources and therefore increase their fitness through the production of more seeds (Watkinson 1982; Aarssen and Clauss 1992).

Evidence was found for the presence of transgressive segregation in both the parental environments. While only two families had a significantly lower fitness than the least fit ecotype (in this case the xeric ecotype) at the xeric site, there were many families which were not significantly different but still fell outside the parental range (Figure 4.3, 4.5). However, while the number of families that did better than the highest parental value is similar to that found in the greenhouse (two families), the number of families with a lower fitness than the least fit parent was much greater at both field sites than it was in the greenhouse. This is consistent with the parental genotypes being adapted to the field environments and, while there are still some extreme phenotypes which can outperform the parental ecotypes in their home environments, there are a substantially higher number of extreme phenotypes which would not be able to outperform the parental ecotypes.

Although in the parental environments there was a larger difference in above ground biomass between the mesic and xeric ecotypes than was found in the greenhouse experiment (Chapter 2), there still seemed to be a great deal of transgressive segregation with a large number of transgressive phenotypes falling outside the parental range. The majority of the extreme phenotypes weighed less than the xeric ecotype, which had the lowest biomass. As above ground biomass and fitness are tightly correlated in the field environment, and a large number of the recombinants had a fitness value lower than the parental ecotypes, this is not unexpected.

This experiment demonstrated that, as in the greenhouse, hybrid vigour which is counteracted by hybrid breakdown is contributing to the fitness of the hybrid offspring in the parental environments. However, contrary to the greenhouse results, transgressive segregation results in a higher number of families exhibiting a lower fitness than the least fit parents in a field environment. Despite this there are still two families which had a higher fitness than the most fit ecotype and may therefore be capable of outperforming the parental ecotypes in the parental environments.

Fitness of F6 generation in parental environments

The primary purpose behind this section of the field experiment was to determine if GxE interactions do occur in the parental environments and if there is any evidence for local adaptation. Although the main effects of family and environment are significant for fitness (Table 4.7), there do not appear to be any GxE interactions in the parental environments. The significant family effects indicate that there are differences in the fitness among families and the significant environment effects indicate that there are differences in how the families respond to the environments. These results are supported by examining the rank order of the families in each of the environments (Figure 4.7). Although there is an increase in fitness between HREC and SFREC, there is not a large change in the rank order of the families. Genetic correlations of less than one between the same trait in two different environments should result in a significant GxE interaction (Via and Lande 1985). Although the genetic correlation was less than one ($r=0.551$, $p<0.01$) in this case it does not seem to result in a significant GxE interaction. When the genetic correlation is high, there will be very little GxE variance (Falconer 1990), therefore, the fact that the correlation between the two environments was significant may

mean there is little GxE variance resulting in a non-significant GxE term. These results are consistent with no local adaptation being present in the parental environments.

Also, should local adaptation be present, each parental ecotype should do better in its respective environment. However, the mesic ecotype outperformed the xeric ecotype at the xeric site. This is interesting given the work which has previously suggested that the five enzyme loci that make up the putative co-adapted gene complexes are locally adapted to the various environments on both a large and small scale (Clegg and Allard 1972). Hutchinson (1982) found that all of the *A. barbata* found around SFREC were of the xeric ecotype. However, it has also been shown that there is a shift in the frequency of genotypes and that the size of the xeric and mesic zones expand and contract based on the level of moisture from year to year (Perez de la Vega *et al.* 1991). The experiment discussed here was performed over one growing season at only two sites. There was a substantial amount of rainfall both in December and in April, and the soil conditions remained wet late into the year. It is possible that if the conditions were unfavourable for the xeric ecotype at both sites, this might lead to it having a poor performance in its own habitat which in turn could contribute to the lack of GxE interactions. It is also possible that selection for a specific genotype occurs at the time of germination. If the xeric ecotype is adapted to germinate under low water conditions while the mesic ecotype is adapted to germinate under slightly wetter conditions it is possible that by germinating the plants prior to planting, selection, which would normally act against the specific ecotypes at the time of germination, could not occur. Numerous environmental factors are going to affect any population at any given time, so whichever one is the most stressful factor at that point in time is the one which will determine the main response of the plant

(Perez de la Vega 1996). Other studies have found that since environmental conditions change from year to year, caution should be used when drawing conclusions from short term studies (Galloway and Fenster 2000; Waser *et al.* 2000). Therefore, while this study does cast some doubt on whether these ecotypes are locally adapted, it is not possible to say from just one field experiment whether these ecotypes are in fact locally adapted to their respective environments. However, the fact that there were GxE interactions in the novel greenhouse environments indicates that the F6 families do sometimes respond differently to different environments and that certain families have the potential to be more adapted to certain environments.

Although above ground biomass does show significant GxE interactions (Table 4.8), the genotypes appear to be responding in the same manner to the two environments in that there is an increase in biomass between HREC and SFREC (Figure 4.8). However, there is a greater change in the rank order of the families than is found for fitness. Also, the correlation between the two environments is not a perfect correlation, which indicates that the response to the environments is different between genotypes. A genetic correlation between environments of less than one indicates that while some of the same genes are involved in the expression of that particular trait, there must also be either different genes in each environment which are influencing the trait or the same genes influencing the trait differently in each environment (Via and Lande 1985; Falconer 1990). Therefore, as the values are not completely independent from one another there must be some of the same genes involved in the expression of above ground biomass in the different environments. As the presence of GxE interactions is consistent with different genotypes responding differently to the environments, it is possible that above

ground biomass is the target of selection in each of the parental environments and that the five allozyme loci, which have been identified as either "mesic" or "xeric" allelic combinations, are associated with gene combinations which are involved in growth rather than actual spikelet number.

The majority of the total variation for both fitness and above ground biomass can be attributed to differences between environments which is consistent with these traits being sensitive to changes in the environments. However, unlike the environments which were created in the greenhouse, where the variance attributable to the genotype x environment interaction was substantially larger than the variance attributable to genotype alone, the field environments showed no difference between the variance attributable to genotype and the variance attributable to the interaction term. This means that in the field, the variation between the different genotypes is no greater than the variation in response to the environment.

Overall, this experiment demonstrated that there are GxE interactions for above ground biomass in the parental environments, indicating that there are differences in how the genotypes respond to the two environments. However, there does not seem to be any GxE interactions for fitness, which again calls into question whether the parental ecotypes are actually exhibiting local adaptation.

Chapter 5

Summary and Conclusions

The importance of hybridization, although now known to be a fairly common occurrence among many organisms, has been debated for decades (Wagner, 1970; Moore 1977; Mayr 1992; Arnold 1997). One view is that hybrid offspring, if they are even viable, will either be sterile or if the hybrid is fertile, will be ill adapted to the environment and will eventually disappear (Wagner 1970). Another view is that hybridization does not necessarily result in an unfit individual but instead may result in recombination upon which selection can act (Arnold 1997; reviewed in Lexer et al. 2003b). A more realistic view may be that hybridization can result in a number of outcomes (Rieseberg and Wendel 1993; Arnold 1997). While some hybridization events may lead to offspring which are inviable or less fit than the parental species, other hybridization events may lead to offspring which are at least as fit if not more so than the parents. These two outcomes may even occur from the same hybridization event, with some of the resulting offspring having a lower fitness than the parents and some exhibiting a fitness that is no different or even higher than the parents. Another aspect of hybridization which has been debated is whether the fitness of the hybrid is independent of the environment. While some argue that selection acts against the hybrid offspring as a result of the disruption of the parental genotypes, others argue that interactions between the environment and the hybrid genotype will determine the outcome of the hybridization event (Arnold 1997). However, as with the question of whether hybrids are by definition fit or unfit, there is no one explanation which will explain the outcome of all hybridization events. Instead, it seems that the relationship between hybrids and the

environment is a combination of both exogenous (interactions between the hybrid genotype and the environment) and endogenous (interaction between parental genomes) selection (Arnold 1997).

The studies reported in this thesis demonstrate that not only can hybridization result in offspring with varying degrees of fitness, but there are also novel environments where hybrid offspring with certain genotypes are capable of outperforming the parental ecotypes. Two studies, one of which was carried out in the greenhouse and one in *A. barbata*'s natural environment, demonstrate that it is possible for both hybrid vigour and hybrid breakdown to occur in the same cross. Evidence from linear contrasts and from the calculation of the net effect of additive, dominance and epistatic gene effects show that there is hybrid vigour, brought about through beneficial dominance effects, present in the early generation hybrids. This hybrid vigour is counteracted by hybrid breakdown, brought about by negative epistatic interactions, which results in an F2 generation with a mean fitness that is no different from the mean fitness of the mid-parent. Had it been possible to produce enough F1 seeds to test this generation against the mid-parent as well, the hybrid vigour which is present should have been more obvious. Although both hybrid vigour and hybrid breakdown are occurring in the same individual, the fact that the F1 generation would be 100% heterozygous would mean that all individuals in this generation would exhibit hybrid vigour. Whereas, in the F2 generation the individuals are heterozygous at 50% of their genes, therefore, the generation mean is lowered by those homozygous individuals which experience no hybrid vigour but only hybrid breakdown. Hybrid vigour, which is counteracted by hybrid breakdown, was found both in a greenhouse environment and in *A. barbata*'s natural habitat. This hybrid breakdown is

strong evidence that there are in fact co-adapted gene complexes in the parental ecotypes which affect fitness. Had this result been found in one environment but not the other, then it is possible that the combination of genotype with the environment was resulting in the lower mean fitness of the F6 generation. However, the fact that this result is repeatable in very different environments clearly indicates that at least some of the fitness exhibited by the parental ecotypes is due to co-adapted gene complexes present in the parental genome and is independent of the environment. The presence of GxE interactions which were found in the novel greenhouse environments however, indicates that the fitness of the hybrids is not completely independent of the environment. These GxE interactions show that the environment the hybrids are grown in does result in a change in fitness depending on the genotype. This supports the theory that hybrid fitness is a combination of both exogenous and endogenous selection.

While it has been hypothesized that the xeric and the mesic ecotypes are locally adapted to their particular habitat and that the five allozyme loci form co-adapted gene complexes which contribute to this local adaptation (Allard *et al.* 1972; Clegg and Allard 1972; Hamrick and Allard 1972; Perez de la Vega *et al.* 1991), no clear evidence for local adaptation was found. There were a large number of families which had a lower fitness than the least fit parent in the parental habitats and this does provide some evidence that the parental gene combinations are adapted to their individual environments. However, the mesic ecotype outperformed the xeric ecotype in the xeric habitat which would not be expected if local adaptation existed. Therefore, there is still no clear evidence that the ecotypes are in fact locally adapted to specific environments. It is possible that the conditions were simply unfavourable for the xeric ecotype in this one growing season.

This study is being repeated over another growing season which should help to answer whether the ecotypes are in fact locally adapted. It is also possible that selection acts at the time of germination and therefore, by germinating the seeds and allowing the seedlings to become established in a greenhouse prior to planting, selection could not act against the most unfit genotype for that environment. Therefore, allowing the seeds to germinate in the parental environments would help in answering this question more fully.

Both the hybrid breakdown which is seen in the later generation hybrids, and the transgressive segregation which results in extreme phenotypes relative to the parents are the result of the formation of recombinant genotypes. While recombination may bring about the disruption of co-adapted gene complexes, which results in a decrease in fitness of the hybrid offspring, the recombination may also result in complementary gene action. If this complementary gene action occurs this in turn will result in some hybrid families experiencing superior (or inferior) fitness relative to the parental trait values. As *A. barbata* is predominately selfing, after the initial out-crossing event this method of reproducing will restrict recombination and will therefore protect any favourable or unfavourable epistatic interactions from being broken up (Allard 1996). The combination of these recombinant genotypes together with the dominant gene effects, which result in the presence of hybrid vigour in the early generation hybrids, may be what is necessary in order for the hybrid offspring to be capable of forming a stabilized hybrid genotype which can subsequently colonize new habitats. The presence of the beneficial dominant gene effects in the early generation hybrids may prevent the hybrids from exhibiting only hybrid breakdown. This hybrid vigour, which results in the F2 generation mean fitness being no different from the mid-parent mean fitness may result in the early generation

hybrids having a high enough fitness that they will not be out-competed by the parental ecotypes. This in turn may allow for self fertilization to occur over a number of generations and ultimately in the production of stabilized recombinant genotypes. While the production of these recombinant genotypes will without a doubt result in some hybrid genotypes which are simply not fit enough to remain in the population, it will also result in some genotypes which are superior to the parental ecotypes in at least one environment. That this scenario could happen is shown by the results of the experiment which tested the later generation hybrids in novel environments. Transgressive segregation, resulting in hybrid genotypes with a fitness superior to that of the parental ecotypes was found not only across all environments in the greenhouse, but also to a small extent in the parental environments. This indicates that there are recombinant genotypes which are capable of outperforming the parental ecotypes in novel environments. Should hybridization between the parental ecotypes occur in *A. barbata*'s natural habitat, it is possible that recombinant genotypes capable of colonizing new habitats would be produced, which would result in the expansion of this species' habitat. Alternatively, it may simply result in increased specialization within the species' current habitat. Also, if one broadly adapted genotype is produced then this genotype may come to predominate throughout the species range.

Overall, this research indicates that hybridization events can result in both hybrid breakdown and hybrid vigour in the same cross. There do appear to be co-adapted gene complexes in the parental ecotypes which contribute to fitness however, there is no clear evidence for local adaptation in these parental ecotypes. As well, the GxE interactions indicate that the fitness of the hybrid offspring is not due to genotype alone but rather a

combination of genotype with the environment. This results in some hybrid families which are capable of outperforming parental ecotypes in novel environments. Finally, transgressive segregation found across different environments, coupled with hybrid vigour in early generation hybrids may ultimately result in some hybrid offspring capable of colonizing new habitats.

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