JILL ARIN JOHNSTON

The Effects of Hybridization on the Physiology and Ecology of Two Louisiana Iris Species, *I. brevicaulis* and *I. fulva*

(Under the Direction of Drs. Lisa Donovan and Michael Arnold)

Mounting evidence suggests that hybridization between species is more common and more important to plant evolution than was previously believed by evolutionary biologists. This body of research addresses the physiological changes that occur in hybrids between *Iris brevicaulis* and *I. fulva*, and the ecological and evolutionary implications of that change. In a series of experiments in the field and greenhouse, I have documented that hybrids live in a different range of habitat than their parent species, and are capable of outperforming parent species in some habitats. The ability to survive in different habitat conditions is attributed to new trait combinations in recombinant hybrid individuals. Explicit tests of flood, drought, and shade tolerance indicate that some hybrid individuals are more tolerant of flooded conditions, but *I. brevicaulis* is superior in dry conditions, and *I. fulva* is superior in the shade. Physiological traits measured on parent species and hybrid individuals were quite similar, and not adequate to explain the observed patterns of relative hybrid fitness. By transplanting both species and several hybrid types into natural habitat in the field, I was that both vegetative and sexual fitness of hybrid individuals was higher than that of both parent species in flooded, *I. fulva*-like habitat, and equivalent to parent species in other habitats tested. Comparisons of seed and adult stage fitness in both field and greenhouse conditions indicate that hybrids and parent species have fairly equivalent fitness at the seed and seedling stage, while adult fitness of hybrid groups is more variable, and more responsive to the environment. Overall, this work suggests that hybrids in natural populations of *I. brevicaulis* and *I. fulva* are important sources of new genetic and phenotypic variation. Hybrids have likely played a large role in shaping the ecology and physiology of the species, and will continue to do so through introgression and ecological divergence of hybrids.

INDEX WORDS: Iris, Louisiana, Hybridization, Hybrid fitness, Plant Evolution, Plant physiology, Plant ecology

THE EFFECTS OF HYBRIDIZATION ON THE PHYSIOLOGY AND ECOLOGY OF TWO LOUISIANA IRIS SPECIES, *I. BREVICAULIS* AND *I. FULVA*

by

JILL ARIN JOHNSTON

B.S., The University of California, Davis, 1996

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2002

THE EFFECTS OF HYBRIDIZATION ON THE PHYSIOLOGY AND ECOLOGY OF TWO LOUISIANA IRS SPECIES, *I. BREVICAULIS* AND *I. FULVA*

by

JILL ARIN JOHNSTON

Approved:

Co-Major Professors:	Lisa Donovan Michael Arnold
Committee:	Shumei Chang James Hamrick Chris Peterson Rebecca Sharitz

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
May 2002

DEDICATION

This work is dedicated to all the people who helped me survive and thrive in graduate school. I want to thank my family for emotional and financial support, my fellow students for conversations both collegial and comical, and my mentors for curbing my scientific whimsy while still validating my view of the world. I most especially want to thank my parents, Linda and Jerry Johnston who still think I studied orchids, but who were always interested and encouraging. I want to thank my co-advisors, Dr. Lisa Donovan and Dr. Michael Arnold, whose very different advising styles kept me on track while giving me the freedom I needed to put together a project that fell very squarely between their two areas of expertise. Thanks to my committee, Jim Hamrick, Chris Peterson, Becky Sharitz, and Shumei Chang. Some of the most important feedback I got during this 5-year process came from my fellow lab mates, most especially Amy Bouck and Jason West, as well as Christina Richards, David Rosenthal, John Burke, Ed Kentner, Renate Wesselingh, Javier Espeleta, Scott Cornman, Joe Williams, and Keirith Snyder. I received invaluable moral support on several occasions from my numerous roommates, especially Alissa Salmore, Rebecca Klaper, and Jenny Cruse. Sara Crockett and Virginia Jin, you were fabulous partners in crime during many adventures that I will remember always. And finally, to Jason for sitting back-to-back with me as we both went through the writing process, I thank you.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

PURPOSE OF STUDY

This study seeks to understand the ecological and evolutionary consequences of gene exchange between two wild, naturally hybridizing species in the Louisiana Iris species complex. Strictly speaking, hybridization between species is a violation of the biological species concept (Dobzhansky 1937), but stable zones of interspecific hybrids have been nearly universally documented (Ellstrand et al. 1996). The author has conducted experiments in field and greenhouse environments to explore the potential for hybrid genotypes to occupy unique ecological niches in a natural hybrid zone. Natural distribution patterns indicated that hybrids were able to survive in habitat that was distinct from that of the parent species. Greenhouse experiments showed that hybrids were very fit in some environmental conditions, but unfit in others, suggesting that environmental context is important to hybrid survival. Experiments in natural conditions found that hybrids are fit both in habitat occupied by their parental species and in unique habitat. It is the belief of the author that the findings of this study provide rigorous evidence that hybridization can generate novel phenotypic traits that affect hybrid fitness, hybrid ecology, and the evolutionary trajectory of hybrids and their parent species.

BACKGROUND

The current study addresses fitness and phenotypes of hybrids between two naturally hybridizing species in both controlled and natural settings. While hybrids have long been viewed by plant breeders as a source of phenotypic innovation, only recently has the evolutionary value of hybrid novelty been explored in natural populations (Bennett and Grace 1990; Grant and Grant 1992; Orians 2000). Because hybrid characters arise from a combination of genes from parental species their expression has historically been categorized as either parent-like or intermediate (Hatfield and Schluter 1999; Rieseberg and Ellstrand 1993; Riley 1938). Recent work has shown that hybridization can also give rise to extreme trait expression (de Vicente and Tanksley 1993; Rieseberg et al. 1999), and that hybrid phenotypes are often mosaics of parental, intermediate, and extreme traits (Arnold 1992; Rieseberg et al. 1999; Rosenthal et al. In press; Schwarzbach et al. 2001). Not all new genetic combinations will be favorable (reviewed in Burke and Arnold 2001), but novel combinations of traits may generate rare hybrid genotypes with high relative fitness, which may be important agents of evolutionary change (Arnold et al. 2001; Burke and Arnold 2001; Lewontin and Birch 1966; Rieseberg et al. 1999; Rieseberg and Ellstrand 1993.)

A variety of theoretical models have been proposed to describe potential hybrid fitness and its effect on hybrid zone evolution (reviewed in Arnold 1997, Hewitt 1988). Each model approaches the relationship between hybrid fitness and environmental context differently. The Hybrid Novelty model (Arnold 1997) predicts that rare individuals in hybrid swarms can have higher fitness than either parental species by possessing unique traits or combinations of traits that are not present in either parental species. The fitness advantage enjoyed by novel hybrid genotypes is predicted to be environment-dependent (Arnold 1997; Barton 2001; Rieseberg et al. 1999). Thus, if a hybrid genotype possesses a trait that permits the tolerance of an extreme habitat (e.g.

flooded areas), the traits that confer that tolerance may not increase, or may even decrease fitness in other environments (Ernst 1990). The alternative models of hybrid zone evolution, the Tension Zone (Barton and Hewitt 1985), Bounded Hybrid Superiority (Moore 1977) and Mosaic models (Harrison 1986; Howard 1986) predict that hybrids are likely to have low fitness due to genetic complications, and have little or no evolutionary impact.

If hybridization is to act as a source or vector of adaptive variation, hybrid genotypes must have higher fitness than their parent species in a subset of habitat types (Anderson and Stebbins 1954; Arnold 1992; Barton and Hewitt 1985; Burke and Arnold 2001; Grant 1981). Physiological tolerance traits will determine the set of habitats in which each hybrid individual can live (Osmond et al. 1987; Tilman 1997; Walter 1979). Environmental context is an important component of the relationship between physiological characters and fitness (Arntz et al. 1998; Harper 1977; Poorter and Lambers 1986; Solbrig 1981). If two parent species are uniquely adapted to different habitat types, hybrids can act as a genetic bridge, allowing adaptive genetic variation to move between species via introgression (Anderson 1949; Arnold 2000). Genetic recombination during hybridization that generates new extreme traits (de Vicente and Tanksley 1993; Rieseberg et al. 1999) which may increase hybrid fitness. Successful hybrid colonization of habitat that is not tolerated by parent species could lead to spatial and genetic isolation, potentially ecological divergence and the eventual founding of a new species (Arnold 1993; Ferguson and Sang 2001; Rieseberg et al. 1999; Rieseberg et al. 1995).

HOW THIS STUDY IS ORIGINAL

Hybridization in the Louisiana Iris species complex has been extensively studied (Anderson 1949; Arnold 1994; Bennett and Grace 1990; Burke et al. 1998; Cruzan and Arnold 1993). None of the previous studies have combined experimental and descriptive techniques to address the ecological and evolutionary potential of hybrids. In this study, ecological and physiological traits were measured, and related to hybrid fitness. Hybrid novelty and extreme trait expression are measured directly and with statistical rigor. Individual environmental factors and their effects on hybrid fitness and trait expression were evaluated. Fitness of experimental hybrids was measured in the field, to capture the complexities of natural selective regimes. In sum, these data reveal high hybrid fitness among Louisiana Iris hybrids, and suggest that hybridization will play (and likely has played) a large role in generating beneficial genetic variation in this species complex.

LITERATURE CITED

Anderson, E. 1949. Introgressive hybridization. Wiley & Sons, New York.

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378-388.

Arnold, M. L. 1992. Natural Hybridization as an Evolutionary Process. Annual Review of Ecology and Systematics 23:237-261.

Arnold, M. L. 1993. Iris-Nelsonii (Iridaceae) - Origin and Genetic Composition of a Homoploid Hybrid Species. American Journal of Botany 80:577-583.

Arnold, M. L. 1994. Natural Hybridization and Louisiana Irises. Bioscience 44:141-147.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Arnold, M. L. 2000. Anderson's paradigm: Louisiana Irises and the study of evolutionary phenomena. Molecular Ecology 9:1687-1698.

Arnold, M. L., E. K. Kentner, J. A. Johnston, S. Cornman, and A. C. Bouck. 2001. Natural hybridisation and fitness. Taxon 50:93-104.

Arntz, A. M., E. H. DeLucia, and N. Jordan. 1998. Contribution of photosynthetic rate to growth and reproduction in Amaranthus hybridus. Oecologia 117:323-330.

Barton, N. H. 2001. The role of hybridization in evolution. Molecular Ecology 10:551-568.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16:113-148.

Bennett, B. D., and J. B. Grace. 1990. Shade Tolerance and Its Effect on the Segregation of 2 Species of Louisiana Iris and Their Hybrids. American Journal of Botany 77:100-107.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the Fitness of Hybrids. Annual Review of Genetics 35:31-52.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998. Hybrid fitness in the Louisiana irises: Analysis of parental and F-1 performance. Evolution 52:37-43.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and Genetic Associations in an Iris Hybrid Zone. Evolution 47:1432-1445.

de Vicente, M. C., and S. D. Tanksley. 1993. Qtl Analysis of Transgressive Segregation in an Interspecific Tomato Cross. Genetics 134:585-596.

Dobzhansky, T. 1937. Genetics and the Origin of Species. Columbia University Press, New York.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Sciences of the United States of America 93:5090-5093.

Ernst, W. H. O. 1990. Ecophysiology of Plants in Waterlogged and Flooded Environments. Aquatic Botany 38:73-90.

Ferguson, D., and T. Sang. 2001. Speciation through homoploid hybridization between allotetraploids in peonies (Paeonia). Proceedings of the National Academy of Sciences of the United States of America 98:3915-3919.

Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. Science 256:193-197.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, San Diego, CA, USA.

Harrison, R. G. 1986. Pattern and Process in a Narrow Hybrid Zone. Heredity 56:337-349.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution 53:866-873.

Hewitt, G. M. 1988. Hybrid Zones - Natural Laboratories for Evolutionary Studies. Trends in Ecology & Evolution 3:158-167.

Howard, D. J. 1986. A Zone of Overlap and Hybridization between 2 Ground Cricket Species. Evolution 40:34-43.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315-&.

Moore, W. S. 1977. Evaluation of Narrow Hybrid Zones in Vertebrates. Quarterly Review of Biology 52:263-277.

Orians, C. M. 2000. The effects of hybridization in plants on secondary chemistry: Implications for the ecology and evolution of plant-herbivore interactions. American Journal of Botany 87:1749-1756.

Osmond, C. B., M. P. Austin, J. A. Berry, W. D. Billings, J. S. Boyer, J. W. H. Dacey, P. S. Nobel, S. D. Smith, and W. E. Winner. 1987. Stress Physiology and the Distribution of Plants. Bioscience 37:38-48.

Poorter, H., and H. Lambers. 1986. Growth and Competitive Ability of a Highly Plastic and a Marginally Plastic Genotype of Plantago-Major in a Fluctuating Environment. Physiologia Plantarum 67:217-222.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg, L. H., and N. C. Ellstrand. 1993. What Can Molecular and Morphological Markers Tell Us About Plant Hybridization. Critical Reviews in Plant Sciences 12:213-241.

Rieseberg, L. H., C. Vanfossen, and A. M. Desrochers. 1995. Hybrid Speciation Accompanied by Genomic Reorganization in Wild Sunflowers. Nature 375:313-316.

Riley, H. P. 1938. A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. American Journal of Botany 25:649-725.

Rosenthal, D. M., A. E. Schwarzbach, L. A. Donovan, O. Raymond, and L. H. Rieseberg. In press. Phenotypic differentiation between three anient hybrid taxa and their parental species. International Journal of Plant Sciences 00:000-000.

Schwarzbach, A. E., L. A. Donovan, and L. H. Rieseberg. 2001. Transgressive character expression in a hybrid sunflower species. American Journal of Botany 88:270-277.

Solbrig, O. T. 1981. Studies on the Population Biology of the Genus Viola .2. The Effect of Plant Size on Fitness in Viola-Sororia. Evolution 35:1080-1093.

Tilman, D. 1997. Mechanisms of Plant Competition *in* M. J. Crawley, ed. Plant Ecology. Blackwell Science, Oxford, U.K.

Walter, H. 1979. Vegetation of the earth in realtion to climate and the eco-physiological conditions. Springer-Verlag, New York, USA.

CHAPTER 2

INTIMATELY LINKED OR HARDLY SPEAKING? THE RELATIONSHIP BETWEEN GENOTYPE AND ENVIRONMENTAL GRADIENTS IN A LOUISIANA IRIS HYBRID POPULATION[1]

---

[1] Johnston, J.A., R. A. Wesselingh, A. C. Bouck, L. A. Donovan, and M. L. Arnold. 2001. *Molecular Ecology.* 10:673-681.
Permission to be reprinted is being requested.

ABSTRACT

Several models of hybrid zone evolution predict the same spatial patterns of genotypic distribution whether or not structuring is due to environment-dependent or -independent selection. In this study, we tested for evidence of environment-dependent selection in an *Iris fulva* X *Iris brevicaulis* hybrid population by examining the distribution of genotypes in relation to environmental gradients. We selected 201 Louisiana Iris plants from within a known hybrid population (80m X 80m) and place them into four different genotypic classes *(I. fulva*, *I. fulva*-like hybrid, *I. brevicaulis*-like hybrid, and *I. brevicaulis*) based on seven species-specific random amplified polymorphic (DNA) markers and two chloroplast DNA haplotypes. Environmental variables were then measured. These variables included percentage cover by tree canopy, elevation from the high water mark, soil pH, and percentage soil organic matter. Each variable was sampled for all 201 plants. Canonical discriminant analysis (CDA) was used to infer the environmental factors most strongly associated with the different genotypic groups. Slight differences in elevation (-0.5m to +0.4m) were important for distinguishing habitat distributions described by CDA, even though there were no statistical differences between mean elevations alone. *I. brevicaulis* occurred in a broad range of habitats, while *I. fulva* had a narrower distribution. Of all the possible combinations, *Iris fulva*-like hybrids and *I. brevicaulis*-like hybrids occurred in the most distinct habitat types relative to one another. Each hybrid class was not significantly different from its closest parent with regard to habitat occupied, but was statistically unique from its more distant parental species. Within the hybrid genotypes, most, but not all, RAPD loci were individually correlated with environmental variables. This study

suggests that, at a very fine spatial scale, environment-dependent selection contributed to the genetic structuring of this hybrid zone.

INTRODUCTION

Defining what makes a species ecologically unique can be a daunting challenge, due to the large number of factors that could be considered (Hutchinson 1957). In addition, species are most often defined by reproductive barriers (Dobzhansky 1937, Mayr 1942, Cracraft 1989, Templeton 1989). This may reveal little or nothing about functional or ecological differences. Phylogenetically related species often occupy similar niches within a community (e.g. Grace and Wetzel 1982). However, if species occur in the same communities, traditional niche theory would predict that they must possess some ecological difference to co-exist (Hutchinson 1959, but see Connell 1978 or Hubbell and Foster 1986).

Rather than attempt to define all aspects of a niche, co-occurring species can be defined in terms of differences from their associates. In systems that are overwhelmingly influenced by a single strong abiotic gradient (i.e. flooding), this factor is the obvious choice to test for its effects on the ecological sorting of species (e.g. *Carex,* Moog 1998; *Ranunculus,* He et al. 1999). In more complex systems, it may be necessary to examine interactions of additional biotic or abiotic factors with the primary gradient to explain niche separation and coexistence (Grace and Wetzel 1982, Hall and Harcombe 1998). In systems without an obvious gradient, it may be difficult to pinpoint individual factors that may be important in determining species distributions. The more factors and interactions that can be evaluated at one time, the more complete the understanding of the niche of the study species (Chapin *et al.* 1987).

Natural species distributions reflect selection by all abiotic and biotic factors at every life history stage (Kotowski *et al.* 1998). Given enough time and dispersal opportunity, reciprocal interactions of genes and environment will lead to predictable patterns of distribution (Salzman 1985, Schupp 1995). Correlating the environment with natural distribution patterns should make it possible to generate hypotheses regarding the most likely factors that determine the types of habitat a species occupies.

The subset of habitats occupied by a species is due to physiological constraints of the species, subtle microsite differences, and natural selection at all life history stages. Bazzaz (1991) coined the phrase “habitat selection” to describe this phenomenon. The environment plays the active role in habitat selection through natural selection on individual plants. The “selected habitat” thus reflects differential, environment-dependent selection on the various genotypes (Bazzaz 1991). Comparison of habitat occupied by multiple species could potentially reveal factors that distinguish the species ecologically.

In situations where interspecific hybridization blurs the species boundary (Arnold 1993, Wang *et al.* 1997), it may become more difficult to recognize characters that distinguish species. Hybrid and introgressed individuals are recombinant, containing genes from both parental species (Anderson and Stebbins 1954). Hybrids may display phenotypes that are intermediate to their parents (Riley 1937), or unique and transgressive (Cruzan and Arnold 1993, Rieseberg *et al.* 1999). If environment-dependent selection is acting on hybrids, then distribution patterns should reflect the genetic make-up of the hybrid individual.

Hybridization within the Louisiana Iris species complex has been studied for over half a century as a phenomenon of systematics (Viosca 1935), genetics (Anderson 1949, Arnold *et al.* 1992), speciation (Randolf 1966, Arnold 1993) and ecology (Bennett and Grace 1990, Cruzan and Arnold 1993). Although rare, F_1 hybrids do form naturally (Arnold 1993, Hodges *et al.* 1996). Previous studies with Louisiana Irises have found environment-dependent selection (Emms and Arnold 1997), evidence that hybrids can occupy unique habitats (Cruzan and Arnold 1993), and the suggestion that in some respects hybrids may be more fit than their parents (Burke *et al.*1998a).

The present study involves two species of Louisiana Iris, *Iris brevicaulis* and *Iris fulva*. *Iris brevicaulis* and *I. fulva* are the most ecologically similar of the Louisiana Iris species, occurring at the edges of bayous and swamps, usually under a tree canopy (Viosca 1935). The two species co-occur in several places in Louisiana, and are difficult to distinguish when not in flower. While sexual reproduction is critical for hybridization, vegetative reproduction is also important for persistence and spread of genotypes once they are established (Emms and Arnold 1997, Burke *et al.* 2000). Hybrids from *I. brevicaulis* X *I. fulva* crosses have been shown to exhibit environment-dependent fitness (Cruzan and Arnold 1993), but the important factors differentiating the types of habitat occupied by *I. fulva* and *I. brevicaulis* have been difficult to quantify.

To gain an understanding of important ecological differences between *I. brevicaulis* and *I. fulva*, we investigated the natural distributions within a hybrid population relative to environmental parameters. Within the general context of testing whether environment-dependent selection was occurring in the study population, we addressed the following questions: 1) Do *I. fulva* and *I. brevicaulis* occupy different types of habitat? 2) Are hybrids found in unique habitat relative to *I. brevicaulis* and *I. fulva*? 3) Is there a single environmental gradient that differentiates the habitats of each genotypic group? 4) Are individual genetic markers within hybrid genotypes associated with environmental factors?

MATERIALS AND METHODS

Study population

Leaf tissue was collected from a population of Louisiana Irises approximately 5 km north of the intersection of state roads 31 and 96 in St. Martin parish, Louisiana. Based on flower colors in previous years, this population was thought to contain *Iris fulva*, *I. brevicaulis*, and hybrid individuals (M. Arnold unpub. data). The plants occurred continuously across a forested slough. The samples were collected within an 80m X 80m area. None of the plants were in flower, and thus there was little to indicate the identity of any individual. Plants were selected haphazardly, at least 1m apart to reduce the likelihood of sampling the same individual twice. Leaf tissue was snap frozen in liquid nitrogen and returned to the University of Georgia for genetic analysis.

Environmental Measurements

It would be impossible to determine all the relevant biotic and abiotic variables influencing habitat distributions in Louisiana irises. We chose to measure four abiotic factors that reflect conditions that affect a range of fundamental plant needs for every plant collected: elevation, percentage cover by canopy trees, soil pH and soil percentage organic matter. Three-dimensional co-ordinates were measured with a TOPCON CTS-2 Total Station, (Topcon America Corporation). A two dimensional map of the individuals in the population was created using X and Y co-ordinates. The Z co-ordinate was used to determine elevation within the slough. Elevation was standardized to zero at the high water mark measured following rainfall in spring 1999.

Light level in the forested slough is influenced by a canopy of second-growth oak, cypress, and pecan trees. Little other vegetation shares the understorey environment during the iris growing season (i.e., December-April). Because sunflecks move across the floor of the forest fragment during the day, an integrated, rather than instantaneous measurement of light condition was deemed preferable. To measure the light environment of each plant, percentage cover by tree canopy was measured with a spherical densiometer (Forest Densiometers) using a modified protocol from Lemmon (1956). The concave mirror of a densiometer has a grid etched into its surface. Percent cover was estimated by calculating the proportion of the corners of squares on the grid that fell in a reflection of tree canopy. Estimates were repeated from four different angles at each plant and then averaged.

Soil cores (2 cm in diameter and 15 cm deep) were sampled immediately adjacent to the rhizome of each plant in the study population. Samples were allowed to air dry in plastic bags. They were then placed at 60°C for 48 hours. Each sample was ground to a fine powder in a ball mill (Spex Certiprep 8000 Mixer/Mill). Subsamples (1-1.5 g) were analyzed for percentage organic matter, measured as mass loss following 20 h in a muffle furnace (Isotemp 550) at 550°C (Parent and Caron 1993). Measurement of pH was done with an Accumet 1003 pH meter (Fisher Scientific) in a 1:2 (soil:water) mixture formed by combining a 3 g soil sub-sample with 6 ml of distilled water (modified from Parent and Caron 1993).

Molecular Analysis

Nuclear and cytoplasmic DNA were isolated from each of the leaf samples using the procedures of Edwards *et al.* (1991). Seven diagnostic random, nuclear markers for *I.*

fulva and *I. brevicaulis* (Cruzan and Arnold (1993) and this study) were screened using random amplified DNA (RAPD) methodology (Williams *et al.* 1990). Each marker was previously shown to be present in either *I. fulva* or *I. brevicaulis*, providing reliable data about the parentage of each individual. The polymerase chain reaction (PCR) amplification protocol for the RAPD analysis utilized one unit of Promega *Taq* polymerase, the Promega *Taq* reaction buffer, 1.9 mM $MgCl_2$, 5 pmol of a single arbitrary primer, and 2 µl of the stock DNA. Amplification was performed in an M.J. Research thermal cycler programmed for 45 cycles of 1 min at 92°C, 1 min at 35°C and 2 min at 72°C. Primers used to generate markers came from the University of British Columbia Biotechnology Lab (see Cruzan and Arnold 1993, except for UBC 502). The RAPD products were separated using 2% agarose gels containing ethidium bromide and then photographed under ultraviolet light.

Chloroplast DNA (cpDNA) haplotypes diagnostic for *I. fulva* and *I. brevicaulis* were screened using the methodology described in Arnold *et al.* (1991) and Arnold *et al.* (1992), with one modification: 5µl of the stock DNA sample were used for the cpDNA amplifications. The cpDNA is maternally inherited in the Louisiana Iris species, and this provides a comparison of nuclear and cytoplasmically inherited markers in hybrid individuals.

Statistical Analyses

Multivariate analysis of the relationship between genotypic classes and environmental variables was performed using Canonical Discriminant Analysis (PROC CANDISC; SAS 1990). These statistics were employed to tease apart the environmental factors most likely to contribute to the difference in habitat occupied by each genotypic group. The statistical procedure created a function that generated a composite variable from the dependent variables (i.e. elevation, cover, soil pH and percentage soil organic matter in this case). This composite canonical variable maximized the differences among a prescribed set of groups (i.e. *I. fulva, I. fulva*-like hybrids, *I. brevicaulis*-like hybrids and *I. brevicaulis*). One orthogonal canonical variable fewer than groups in the analysis was generated. Correlation of canonical variables with each environmental variable suggested which environmental factors most likely contribute to habitat differentiation among genotypic classes. Mahalonobis distance analysis was used for multivariate means comparison among genotypic classes (Johnson 1998).

Prior to statistical analyses, the scale of each variable measured was standardized to become a dimensionless value falling between zero and one (Johnson 1998). This prevented interference by differences in units, magnitude, or dimension of variables. Ten data points were removed from the data set in order to satisfy the assumption for CDA that requires data to have a multivariate normal distribution. The ten points removed fell two standard deviations beyond the mean of at least one variable, and had a large skewing effect on the analysis. The most severe outliers were removed one at a time, until the discriminant functions stabilized. Removal of outlying data points generated a more conservative estimate of environmental influence than if all data points had been included in the analysis.

In a second set of analyses, only hybrid genotypes were analyzed to look for ecological associations of individual markers. Canonical discriminant analysis was performed for each marker in order to determine whether individual loci within hybrid individuals showed signs of association with environmental variables.

RESULTS

Ecological associations of genotypic classes

Examining genotypic class means of environmental variables did not reveal any meaningful trends (Table 2.1). It was thus necessary to examine the results of the multivariate analysis to gain an understanding of how the genotypic classes differed in their habitat characterization.

Canonical discriminant analysis suggested the four genotypic classes occurred in somewhat different habitats (Figure 2.1). *Iris brevicaulis* occupied the full range of both canonical variables, while the other three genotypic classes were more restricted in their distribution across habitat types. The means of each genotypic class (Figure 2.1) showed separation of *I. fulva* and *I. fulva*-like hybrids, from *I. brevicaulis* and *I. brevicaulis*-like hybrids along canonical variable 1 (Can1). Can2 further separated *I. brevicaulis*-like hybrids from the remaining classes. *Iris brevicaulis*-like hybrids and *I. fulva*-like hybrids were the most different groups as described by Can1 and Can2. Mahalanobis distance compared means of each pair of genotypic classes. *I. fulva* occurred in significantly different habitat compared to *I. brevicaulis* (P = 0.0006). In addition, the hybrid classes occupied different habitats relative to one another (P = 0.0001), and the species they are least like genetically (P = 0.0025, 0.0001; Table 2.2). Though not significant, there was also a trend for each species to be ecologically unique from its most similar hybrid.

The canonical discriminant analysis describes 19% of the variation in the distributions of the four genotypic classes. Within the variation described by canonical analysis (Table 2.3), Can1 was responsible for 77% of the separation of these four groups. Can2 accounted for 14% of the separation. The third canonical variable did not

Table 2.1. Genotypic class means of four environmental variables measured for canonical discriminant analysis of habitat preference in a Louisiana Iris hybrid zone. Values reported are means ± SD.

Genotypic Class	Elevation (m)	Tree Cover (%)	Soil pH	Soil organic matter (%)
I. brevicaulis	-0.020 ± 0.065	90.5 ± 3.9	6.67 ± 1.55	5.81 ± 0.35
I. brevicaulis-like hybrids	0.009 ± 0.106	88.6 ± 4.7	6.65 ± 1.40	5.91 ± 0.34
I. fulva-like hybrids	-0.070 ± 0.103	87.5 ± 3.9	7.67 ± 2.00	5.69 ± 0.23
I. fulva	-0.080 ± 0.035	88.7 ± 4.2	6.81 ± 1.65	5.80 ± 0.33

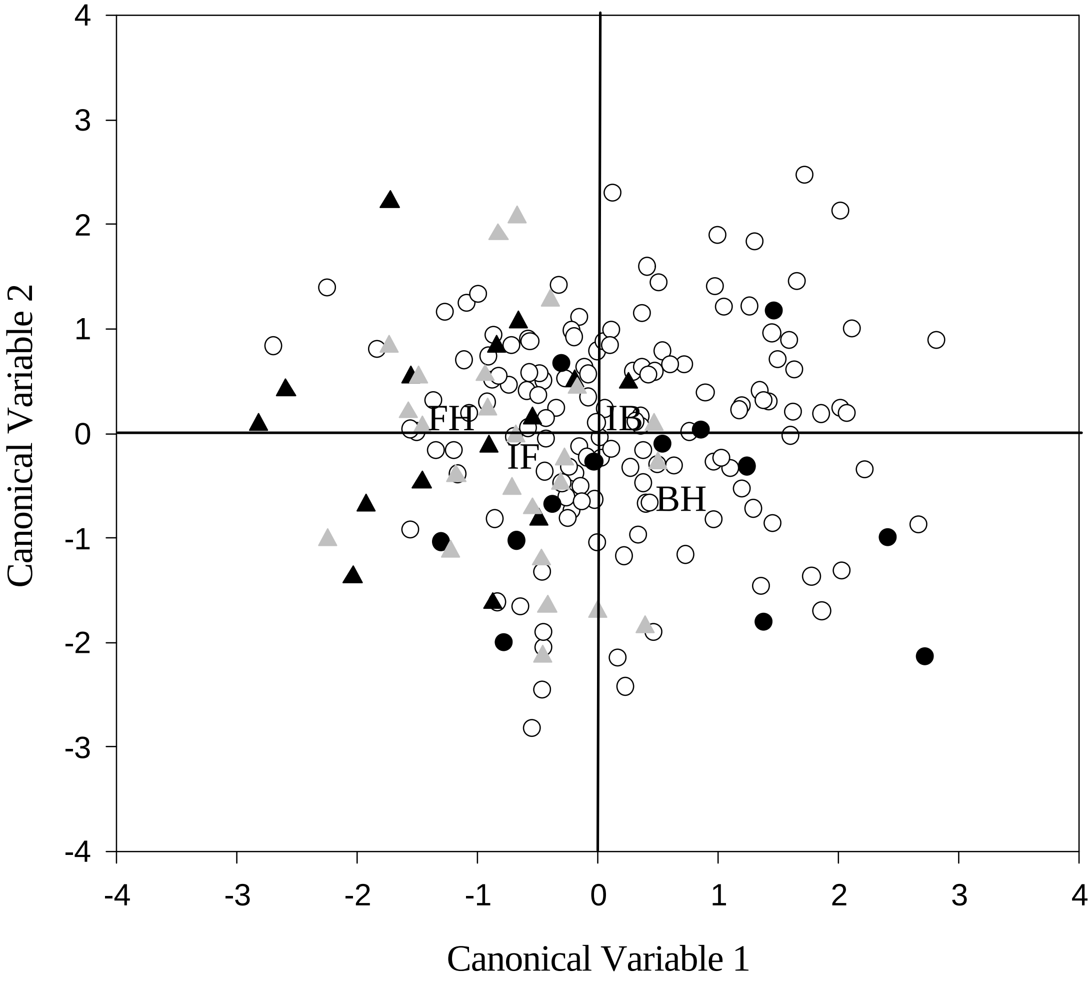

Figure 1.1. First and second canonical axes generated by canonical discriminant analysis of environmental associations in four genotypic classes of Louisiana Iris. Elevation, canopy cover, soil pH, and soil organic matter were measured for each plant included in this analysis (N = 191 plants). *Iris brevicaulis* and *I. brevicaulis*-like hybrids are represented by open and closed circles, respectively. *Iris fulva* and *I. fulva*-like hybrids are represented by gray and black triangles, respectively. Means for each class are denoted by the following: IB = *I. brevicaulis*, BH = *I. brevicaulis*-like hybrids, FH = *I. fulva*-like hybrids, and IF = *I. fulva*.

Table 2.2: Probability that Mahalanobis distance indicates significant levels of habitat distinction between genotypic classes in a natural hybrid population of Louisiana Irises. P-values are shown for all pairwise comparisons. Statistical significance is indicated as follows: “**” = 99%, “***” = 99.9%, and “n.s.” = not significant.

Genotypic Class	*I. brevicaulis*	*I. brevicaulis*-like hybrids	*I. fulva*-like hybrids	*I. fulva*
I. brevicaulis	1.0000	0.0788 (n.s.)	0.0001***	0.0006***
I. brevicaulis-like hybrids		1.0000	0.0001***	0.0025**
I. fulva-like hybrids			1.0000	0.1166 (n.s.)
I. fulva				1.0000

Table 2.3. Canonical discriminant analysis for environmental associations in four genotypic classes of Louisiana Iris (*I. brevicaulis*, *I. brevicaulis*-like hybrids, *I. fulva*-like hybrids, *I. fulva*). Discriminant coefficients describe the discriminant functions generated from all environmental variables to maximize separation between genotypic classes. The amount of variation each canonical variable describes is calculated from the eigenvalue. P-values show probability that each canonical variable describes a significant amount of variation.

	Unstandardized discriminant coefficients							Standardized discriminant coefficients			
Canonical Variable	Elevation	Cover	Soil Organics	Soil pH	Eigen-value	% con-tribut.	P<0.05	Eleva-tion	Cover	Soil Organics	Soil pH
Can1	5.907	-0.922	-0.921	-0.349	0.2370	77.48	0.0001	1.138	-0.200	-0.175	-0.071
Can2	-0.585	5.1859	1.987	-1.987	0.0422	13.80	0.0499	-0.113	1.047	0.378	-0.401
Can3	3.310	-2.055	4.700	-1.506	0.0267	9.73	0.0860	0.675	-0.418	0.894	-0.308

explain a statistically significant amount of the separation. Each canonical variable was strongly correlated with only one of the environmental factors (Table 2.4). Can1 was tightly correlated with elevation (r = 0.977), Can2 was associated with percentage canopy cover (r = 0.811), and Can3 was dominated by percentage soil organic matter (r = 0.817). Contrary to the simple means of each environmental factor, the canonical means for each genotypic class suggested that *I. brevicaulis* and *I. brevicaulis*-like hybrids did tend to live in higher elevation sites than *I. fulva*, and especially *I. fulva*-like hybrids (Figure 2.1). Sorting along the Can2 axis suggests that *Iris brevicaulis*-like hybrids may grow in areas with slightly more open canopies than the other three groups. The hybrid classes lived in a similar range of habitats to *I. fulva*, and a slightly narrower range than *I. brevicaulis.*

Ecological associations of individual markers

When hybrid genotypes were analyzed separately with canonical discriminant analysis, several individual markers correlated with unique environmental factors. This correlation is summarized by Can1 (Table 2.5). In contrast, three markers, L180, F165A, and B502-2 did not show significant separation when multivariate discriminant analysis was performed. All remaining markers showed significant, but slightly different, associations with environmental variables. In all cases, elevation appeared to be the major factor. Soil percentage organic content and pH were moderately correlated with all canonical variables that described habitat differences. Canopy cover was the least important environmental factor in determining habitat preferences. The canonical variable constructed for each marker was slightly different. However, since all markers were similarly correlated with the environmental variables, means of canonical variables for the *I. brevicaulis* and *I. fulva* forms of the markers were also similar. The exception is

Table 2.4. Correlation of canonical variables generated to describe habitat associations of a hybrid population of Louisiana Irises with each of four environmental variables. A higher absolute value for correlation denotes a larger contribution by that environmental variable.

Canonical Variables	Environmental Variables			
	Elevation (m)	Cover (%)	Soil Organics (%)	pH
Can1	0.977	0.356	-0.453	0.356
Can2	0.053	0.811	0.168	-0.293
Can3	0.036	-0.397	0.817	-0.276

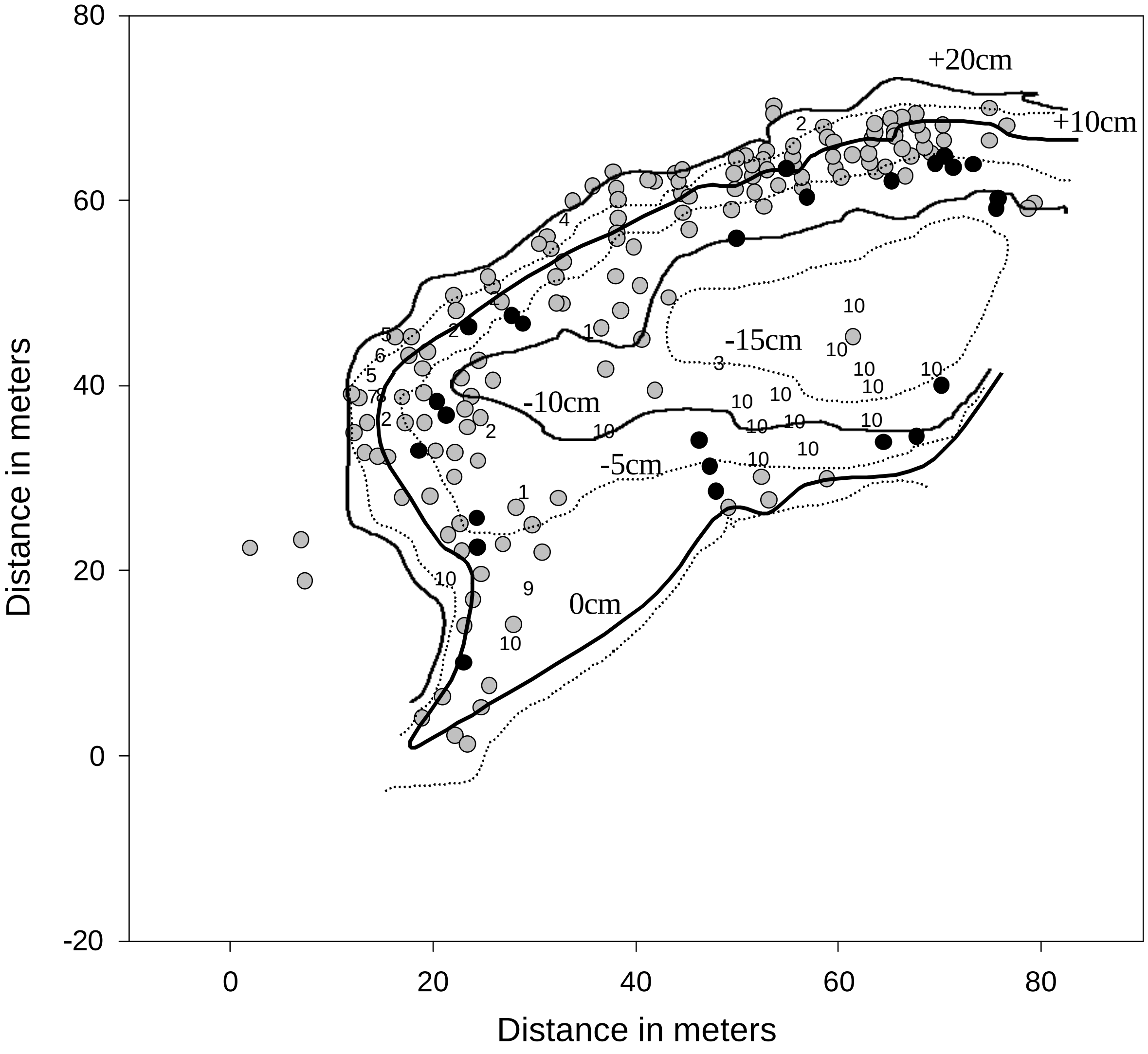

Figure 2.2. Spatial distribution of Louisiana Iris genotypes in a natural population of *I. brevicaulis, I. fulva*, and their hybrids. *Iris brevicaulis* and *I. fulva* are represented by gray and black circles, respectively. Numbers denote unique hybrid genotypes. 1-8 are *I. brevicaulis*-like hybrids, 9 and 10 are *I. fulva*-like hybrid genotypes.

Table 2.5. Canonical discriminant analysis of environmental associations of individual RAPD loci in hybrid Louisiana Irises. Frequency of a given marker (N), correlation of environmental factors with the canonical variable generated, probability that separation of groups is achieved, and the means of the canonical variable for the *I. brevicaulis* (B) and *I. fulva* (F) forms of each marker are given.

	N		Correlation with environment				Discrimination Probability	Means of canonical variable	
Marker	B	F	Elevation	Cover	Organics	pH	P < 0.05	B	F
L180	6	2	0.773	0.656	-0.132	0.730	n.s.	0.323	-0.088
F154	19	9	0.915	0.158	-0.755	0.556	0.007**	-0.591	1.247
F165A	11	17	0.876	0.107	-0.555	0.779	n.s.	0.812	-0.525
F169B	12	16	0.879	0.214	-0.864	0.407	0.002**	1.131	-0.848
B156A	13	15	0.899	0.028	-0.637	0.522	0.005**	0.971	-0.842
B502-1	12	16	0.754	-0.202	-0.646	0.401	0.009**	0.967	-0.725
B502-2	12	16	0.872	-0.004	-0.672	0.530	n.s.	0.719	-0.539
cpDNA	13	15	0.899	0.028	-0.637	0.522	0.005**	0.971	-0.842

F154, which had the most unique environmental associations. F154 had the strongest correlation with soil organic matter (r = -0.755), and appeared to have an association with elevation that was the inverse of all other markers (Table 2.5).

DISCUSSION

Genotypic classes and habitat associations

There were significant differences between the habitats occupied by *Iris brevicaulis* and *I. fulva*. On average, *I. fulva* occurred at lower elevations in deeper cover than *I. brevicaulis*, but with some overlap. These findings generally support previous descriptions of *I. brevicaulis* and *I. fulva* habitat differences (Viosca 1935, Cruzan and Arnold 1993). In this population, *I. brevicaulis* exists in a wide range of possible habitats. In contrast, *I. fulva* occupies a narrow range of habitat, as described by canonical variable 1 (Can1). Our analysis thus suggests that *I. fulva* is somehow restricted to lower elevation sites. Similar patterns have been observed in *Typha* and *Ranunculus* (Grace and Wetzel 1982, He *et al.* 1999), and can be explained by competitive displacement from the more optimal (i.e. drier) habitat. In these systems, higher flood tolerance in one species permits coexistence with another competitive dominant.

In the Louisiana Iris system it was assumed that the range for *I. brevicaulis* did not include areas under standing water due to low flood tolerance. The previously reported restriction of *I. fulva* to more flooded, presumably less optimal, habitat (Viosca 1935, Cruzan and Arnold 1993) could have been explained by higher flood tolerance in *I. fulva*, and weak competitive ability against *I. brevicaulis* on drier ground. Unexpectedly, the elevation data in the current study indicate that *I. brevicaulis* can live quite successfully in areas that are regularly under standing water. Possibly, *I. fulva* and *I.*

brevicaulis have similar flooding tolerance, but *I. fulva* may not be tolerant of dry conditions, limiting it to lower elevation sites. We did not find sharp zonation between the two species, as was previously reported, and will address competitive ability specifically in future studies.

There are several possibilities that might explain the appearance of *I. brevicaulis* in habitat that was previously believed to be too wet for their survival (Viosca 1935). First, our assumption of little physiological tolerance to flooding by *I. brevicaulis* was based only on previous descriptive evidence, and may have been incorrect. Second, encroachment into lower elevations may have occurred by rhizome growth during the dry year preceding our study. Finally, this population is in a slough that is cut off hydrologically from the nearby bayou, which may reduce the number or duration of flooding events during the growing season. Additional factors such as reduced competitive ability may explain why *I. brevicaulis* is less frequently found in the lower elevation habitats in other populations (Cruzan and Arnold 1993).

I. brevicaulis-like hybrids and *I. fulva*-like hybrids occupy the most distinct habitat types of any pairwise comparison. *Iris fulva*-like hybrids occur more often at lower elevation in slightly deeper cover than the *I. brevicaulis*-like hybrids. Each hybrid class had a few individuals in habitat on the fringes of the canonical distribution. This result may indicate that some recombinant individuals are able to exist in habitat that is more extreme or unique from that of the parental species. However, both hybrid classes also have individuals at the center of the canonical distribution, indicating that they are found in habitat that is similar to that of the parental species. Thus, the generalization that hybrids, on average, occur in marginal or ecotonal habitat (Endler 1977, Moore 1977) does not hold in this population.

Hybrid genotypes occupy a broad range of possible habitats, but seem largely to overlap with their closest parental species. Individual hybrid genotypes do occur on the periphery of the canonical distribution, suggesting that they could potentially occupy unique habitat types. Two of the 10 outlying points which were removed from the analysis were hybrid genotypes, the other eight were *I. brevicaulis,* demonstrating that hybrid genotypes are among those genotypes that occur in habitats that fall more than two standard deviations from the norm. Visual interpretation of the data suggests that *I. fulva* is more similar to *I. fulva*-like hybrids than *I. brevicaulis* is to *I. brevicaulis*-like hybrids. However, the apparent differences are not supported by the statistics (Table 2.2). In a previous study, across a more diverse range of habitat, Cruzan and Arnold (1993) found that *I. fulva*-like hybrids were statistically different from *I. fulva*, while *I. brevicaulis*-like hybrids were not significantly different from *I. brevicaulis.* The current study found that, on average, neither hybrid class occurred in habitat that was unique from its closest parent.

Interpretation of the means of these genotypic classes should include the caveat that the *I. brevicaulis*-like hybrid class encompasses more variation than the *I. fulva*-like hybrids. The *I. fulva*-like hybrids were comprised of two RAPD genotypes with one *I. brevicaulis* marker and seven *I. fulva* nuclear and cytoplasmic markers. It is likely that these individuals are advanced generation hybrids. The *I. brevicaulis*-like hybrids are made up of eight unique RAPD genotypes that contain one to four *I. fulva* markers, and therefore four to seven *I. brevicaulis* nuclear and cytoplasmic markers. There are *I.*

brevicaulis-like individuals that represent potential early generation hybrids, one that may be an F_1. Therefore, differences in average response to environmental variables by hybrid classes may have been due, in part, to variation in genotypic diversity.

Ideally, there would be enough replication of each hybrid genotype to statistically analyze each one as its own class. However, several genotypes occur only once, prohibiting such an analysis. By grouping hybrids into two types, *I. brevicaulis*-like and *I. fulva*-like, we believe we have separated the individuals into biologically relevant groups. All the members of a genotypic class share a cpDNA haplotype and the majority of their nuclear alleles. While having two classes of hybrids does not account for all of the variation that is likely found among hybrid individuals, we feel it is an improvement over one catch-all hybrid category which is commonly used in natural hybrid populations (see Arnold and Hodges 1995, Arnold 1997; for a discussion and references).

Individual Loci and Habitat Associations

A majority of the RAPD loci, and the cpDNA haplotypes, display associations with environmental conditions. The fact that markers show environmental association strengthens the argument that environment dependent selection is at work in this hybrid population. Some models of hybrid zone evolution assume that selection against hybrids is environment independent and due largely to complications from recombination between divergent genotypes (e.g., Barton and Hewitt 1985). Previous studies of the Louisiana Irises have suggested that selection in hybrid zones is dependent on environmental factors (Arnold and Bennett 1993, Cruzan and Arnold 1993, Emms and Arnold 1997, Burke *et al.* 1998a,b). This conclusion is consistent with environment dependent hybrid zone models (Endler 1977, Moore 1977, Harrison 1986, Howard 1986, Arnold 1997).

Clearly, our present results suggest an effect of environment dependent selection. However, the pattern is not entirely consistent with the Mosaic model (Rand and Harrison 1989) because there is overlap in the distributions of the genotypic classes, rather than clusters that mirror habitat patchiness. The habitat in which we sampled might better be described by subtle environmental gradients overlaid by microsite differences, as opposed to a mosaic of well defined habitat patches. In addition, because hybrids are scattered throughout the parental species' distributions (Figure 2.2), they do not appear to be marginalized to ecotonal habitat (Moore 1977) as would be expected from the Bounded Hybrid Superiority model.

There is a large range of habitat occupied by the hybrid classes, including some areas that are on the fringes of the canonical distribution of all individuals. It thus seems more appropriate to invoke the Hybrid Novelty model to describe evolution in this hybrid population (Arnold 1997). This model allows for a large variation in hybrid phenotype and fitness. Hybrid phenotypes may be intermediate, similar, or transgressive compared to the parental species (Arnold 1997, Rieseberg *et al.* 1999). If habitat occupied by a hybrid individual can be taken as an indicator of its ecological adaptations, then hybrids in this population have the capability to exhibit numerous parental trait combinations (Anderson 1948).

The current study was conducted on a very fine scale both spatially and in terms of the magnitude of environmental variables measured. By minimizing within site heterogeneity, relevant information about differences in habitat associations of each genotypic class is increased. The observation of significant statistical separation of genotypic groups, across such a fine gradient, is consistent with strong environment-dependent selection. Field and greenhouse studies are underway to investigate experimentally the effects of individual gradients on the fitness of Louisiana Irises and their hybrids.

LITERATURE CITED

Anderson E (1948) Hybridization of the habitat. *Evolution* **2**, 1-9.

Anderson E (1949) *Introgressive Hybridization*. John Wiley and Sons, New York.

Anderson E, Stebbins GL (1954) Hybridization as an evolutionary stimulus. *Evolution* **8**, 378-388.

Arnold ML (1993) *Iris nelsonii* (Iridaceae): origin and genetic composition of a homoploid hybrid species. *American Journal of Botany* **80**, 577-583.

Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford University Press, New York.

Arnold ML, BD Bennett (1993) Natural Hybridization in Louisiana Irises: Genetic Variation and Ecological Determinants. In *Hybrid Zones and the Evolutionary Process*. (ed. Harrison RG) pp. 115-139. Oxford University Press, Oxford.

Arnold ML, Buckner CM, Robinson JJ (1991) Pollen mediated introgression and hybrid speciation in Louisiana Irises. *Proceedings of the National Academy of Sciences USA* **88**, 1398-1402.

Arnold ML, Robinson JJ, Buckner CM, Bennett BD (1992) Pollen dispersal and interspecific gene flow in Louisiana Irises. *Heredity* **68**, 399-404.

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16**, 113-148.

Bazzaz FA (1991) Habitat selection in plants. *American Naturalist* **137**, S116-S130.

Bennett BD, Grace JB (1990) Shade tolerance and its effect on the segregation of two species of Louisiana Iris and their hybrids. *American Journal of Botany* **77**, 100-107.

Burke JM, Carney SE, Arnold ML (1998a) Hybrid fitness in the Louisiana Irises: Analysis of parental and F_1 performance. *Evolution* **52**, 37-43.

Burke JM, Voss TJ, Arnold ML (1998b) Genetic interactions and natural selection in Louisiana Iris hybrids. *Evolution* **52**, 1304-1310.

Burke JM, Bulger MR, Wesselingh RA, Arnold ML (2000) Frequency and spatial patterning of clonal reproduction in Louisiana Iris hybrid populations. *Evolution* **54**, 137-144.

Chapin FS, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. *BioScience* **37**, 49-57.

Connell JH (1978) Diversity in tropical rain forests and coral reefs. *Science* **199**, 1302-1310.

Cracraft J (1989) Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In *Speciation and its Consequences* (eds. Otte D, Endler JA) pp. 28-59. Sinauer, Sunderland, MA.

Cruzan MB, Arnold ML (1993) Ecological and genetic associations in an Iris hybrid zone. *Evolution* **47**, 1432-1445.

Dobzhansky T (1937) *Genetics and the Origin of Species*. Columbia University Press, New York.

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* **19**, 1349.

Emms SK, Arnold ML (1997) The effect of habitat on parental and hybrid fitness: reciprocal transplant experiments with Louisiana Irises. *Evolution* **51**, 1112-1119.

Endler JA (1977) *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, NJ.

Grace JB, Wetzel RG (1982) Niche differentiation between two rhizomatous plants species: *Typha latifolia* and *Typha angustifolia*. *Canadian Journal of Botany* **60**, 46-57.

Hall RBW, Harcombe PA (1998) Flooding alters apparent position of floodplain sapling on a light gradient. *Ecology* **79**, 847-855.

Harrison RG (1986) Pattern and process in a narrow hybrid zone. *Heredity* **56**, 337-329.

He JB, Bogemann GM, van de Steeg HM, Rijnders JGHM, Voesnek LACJ, and Blom CWPM (1999) Survival tactics of *Ranunculus* species in river floodplains. *Oecologia* **118**, 1-8.

Hodges SA, Burke JM, Arnold ML (1996) Natural formation of iris hybrids: experimental evidence on the establishment of hybrid zones. *Evolution* **50**, 2501-2509.

Howard DJ (1986) A zone of overlap and hybridization between two ground cricket species. *Evolution* **40**, 34-43.

Hubble SP, Foster RB (1986) Biology, chance, and history and the structure of tropical rain forest tree communities. In *Community Ecology* (eds. Diamond J, Case TJ) pp. 314-329. Harper and Row, New York.

Hutchinson GE (1957) Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology* **22**, 415-427.

Hutchinson GE (1959) Homage to Santa Rosalia; or, Why Are There So Many Kinds of Animals? *The American Naturalist* **93**, 145-159.

Johnson D (1998) *Multivariate Statistics for Data Analysts.* Duxbury Press, Pacific Grove.

Kotowski W, van Diggelen R, Kleinke J (1998) Behavior of wetland plant species along a moisture gradient in two geographically distant areas. *Acta Botanica Neerlandica* **47**, 337-349.

Lemmon PE (1956) A spherical densiometer for estimating forest overstory density. *Forest Science* **2**, 314-320.

Mayr E (1942) *Systematics and the Origin of Species*. Columbia University Press, New York.

Moog PR (1998) Flooding tolerance of *Carex* species. I. Root structure. *Planta* **207**, 189-198.

Moore WS (1977) An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology* **52**, 263-277.

Parent LE, Caron J (1993) Physical Properties of Organic Soils. In: *Soil Sampling and Method of Analysis.* (ed. Carter MR). Pp. 441-471. Canadian Society of Soil Science.

Rand DM, Harrison RG (1989) Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* **43**, 432-449.

Randolf LF (1966) *Iris nelsonii*, a new species of Louisiana Iris of hybrid origin. *Baileya* **14**, 143-169.

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. *Heredity* **83**, 363-372.

Riley HP (1937) A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. *American Journal of Botany* **25**, 727-738.

Salzman AG (1985) Habitat selection in a clonal plant. *Science* **228**, 603-604.

SAS (1990) User's guide, Version 6, 4th edition. SAS Institute Inc. Cary, NC.

Schupp EW (1995) Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* **82**, 399-409.

Templeton AR (1989) The meaning of species and speciation: a genetic perspective. In *Speciation and its Consequences* (eds. Otte D, Endler JA) pp. 3-27. Sinauer, Sunderland, MA.

Viosca P (1935) The Irises of southeastern Louisiana: a taxonomic and ecological interpretation. *Bulletin of the American Iris Society* **57**, 3-56.

Wang H, McArthur ED, Sanderson SC, Graham JH, Freeman DC (1997) Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae) IV. Reciprocal transplant experiments. *Evolution* **51**, 95-102.

Wijesinghe DK, Hutchings MJ (1996) Consequences of patchy distribution of light for the growth of the clonal herb *Glechoma hederacea*. *OIKOS* **77**, 137-145.

Wijesinghe DK, Hutchings MJ (1997) The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: An experimental study with *Glechoma hederacea*. *Journal of Ecology* **85**, 17-28.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-6535.

CHAPTER 3

FITNESS AND PHYSIOLOGICAL PERFORMANCE IN RESPONSE TO SHADE AND DROUGHT IN TWO LOUISIANA IRIS SPECIES AND BACK CROSS HYBRIDS[1]

---

[1] J. A. Johnston, M.L. Arnold, J. B. West and L. A. Donovan. To be submitted to *International Journal of Plant Sciences*

ABSTRACT

Hybridization has the potential to generate individuals with physiological traits and ecological niches that differ from the parent species. *Iris brevicaulis* and *Iris fulva* are two naturally hybridizing species whose habitat distributions differ in terms of drought and shade. We measured physiological traits (gas exchange, biomass allocation, specific leaf area, leaf area ratio, leaf toughness, and non-destructive growth rate) and fitness components (biomass and number of ramets) of *Iris brevicaulis, I. fulva*, and their reciprocal first generation backcross hybrids (BCIB and BCIF) in four experimental environments (sun/damp, shade/damp, sun/dry, shade/dry). Among hybrid individuals, we found evidence of transgressive trait expression for every physiological trait and one of the fitness components. The physiological character with the most extreme transgressive expression was number of leaves (a growth estimator), which showed both positive (in sun) and negative (in shade) transgressive expression by BCIB hybrids. Expression of many characters, such as photosynthetic rate, leaf area ratio, specific leaf area, total biomass and biomass allocation showed some degree of environmental dependence, but both species and hybrid classes tended to respond to the environment in a similar manner. Fitness components showed that hybrid fitness was environment-dependent, and that BCIB hybrids were the fittest genotypic class in the sun/damp treatment. In sum, these data provide support for transgressive, environment-dependent physiological character expression in Louisiana Iris hybrids, and show environmentally dependent hybrid fitness.

INTRODUCTION

The potential contribution of hybridization to the creation and evolution of species has long been undervalued (Barton and Hewitt 1985; Burke and Arnold 2001; Mayr 1942). Because hybrid characters are the product of gene combinations from parental species, hybrid trait expression has traditionally been categorized as either parent-like or intermediate (Hatfield and Schluter 1999; Rieseberg and Ellstrand 1993; Riley 1938). Recent work has shown that hybridization can generate extreme or transgressive trait variation and produce phenotypes that are mosaics of parental, intermediate, and transgressive traits (Arnold 1992; Rieseberg et al. 1999; Rosenthal et al. 2002; Schwarzbach et al. 2001). Not all new genetic combinations will be favorable (reviewed in Burke and Arnold 2001), but transgressive character expression and novel trait combinations may generate rare hybrid genotypes with high relative fitness that have the potential to be important agents of evolutionary change (Arnold et al. 2001; Burke and Arnold 2001; Lewontin and Birch 1966; Rieseberg et al. 1999; Rieseberg and Ellstrand 1993).

The environment is likely to play a role in the success of hybrid genotypes (Arnold 1997; Hewitt 1988). Physiological tolerance traits will determine the abiotic limits within which each hybrid genotype can succeed (Osmond et al. 1987; Tilman 1997; Walter 1979). If hybrids arise from crosses between two species that are adapted to different habitats, the resulting genotypes may contain mixtures of physiological traits from each parent (Burke and Arnold 2001). The relationship between physiological characters and fitness is highly dependent on environmental context (Arntz et al. 1998; Harper 1977; Poorter and Lambers 1986b; Solbrig 1981b). Novel combinations of physiological traits may result in hybrids that are suited to different habitat than either parent, including some environments that neither species can inhabit (Arnold 1993; Rosenthal et al. 2002). Furthermore, novel hybrid traits may only be expressed in some environmental conditions (Weber and D'Antonio 1999). Many ecosystems exhibit fine-scale habitat heterogeneity (e.g. wetlands-Huenneke and Sharitz 1986; Santiago et al. 2000; Snow and Vince 1984), presenting a variety of ecological opportunities, increasing the chances for hybrid genotypes to reach their full fitness potential. Successful hybrid colonization of habitat not tolerated by either parent species, could lead to spatial and genetic isolation, ecological divergence and the eventual founding of a new species (Arnold 1993; Ferguson and Sang 2001; Rieseberg et al. 1999; Rieseberg et al. 1995).

The Louisiana Iris species complex lends itself to questions regarding evolutionary ecology of hybrids. There are four species in the group that all co-occur in southern Louisiana (Arnold 1994; Viosca 1935). They are found in different types of wetlands along the bayous and swamps of southern Louisiana, and can hybridize where they come in contact (Anderson 1949; Arnold 1994; Hodges et al. 1996). Two species, *Iris brevicaulis* and *I. fulva* share similar habitat along the edges of bayous (Cruzan and Arnold 1993; Viosca 1935). Water depth fluctuates widely and tree cover is patchy, resulting in habitat that is heterogeneous on a very fine scale (Cruzan and Arnold 1993; Johnston et al. 2001b). *Iris brevicaulis* occurs more often in drier, sunnier habitat, while *I. fulva* is found in wetter, shadier areas (Cruzan and Arnold 1993; Johnston et al. 2001b). Hybrid genotypes can be interspersed with parent species or live in extremely sunny, dry, or wet habitat patches (Johnston et al. 2001b). Both field and greenhouse studies suggest that physiological tolerance and fitness of hybrids can be unique from both parent species (Johnston et al. 2001a; Johnston et al. 2001b).

The genetic consequences of hybridization in the Louisiana Iris species complex have been well-studied (Anderson 1949; Arnold 1993; Arnold 1994; Burke et al. 1998a; Burke et al. 1998b). Louisiana Iris hybrids have high viability (Emms et al. 1996) and reproductive fitness in a controlled greenhouse environment (Burke et al. 1998a). In this study, we build on previous work by examining physiological traits of *I. brevicaulis*, *I. fulva*, and hybrids, linking traits to fitness and examining the effects of environmental context. We specifically addressed three questions. First, do *I. brevicaulis, I. fulva*, and their hybrids have measurably different physiological traits of ecological importance? Second, does trait expression change across environments? Third, we asked whether hybrids are ever more fit than parental species, and whether it depends on environmental context? Measuring the response of hybrid genotypes to several experimental environments will inform our understanding of the abiotic factors that affect hybrid fitness in this system and the importance of environment dependent trait expression for the evolution of plant physiological traits in this hybrid system.

METHODS

Plant material

Two plant species, *Iris brevicaulis* and *I. fulva*, and two types of hybrid cross were used in this experiment. Rhizomes of both species were collected from the field in 1995 (*I. brevicaulis* - St. Martin Parish, Louisiana, USA, *I. fulva* – Terrebonne Parish, Louisiana, USA) and maintained in the University of Georgia Botany greenhouse (Athens, GA, USA). Hereafter, *I. brevicaulis* and *I. fulva* will be referred to as IB and IF, respectively. IB by IF crosses were made in 1996 to produce F_1 hybrids. Pollen from F_1 hybrids was put back onto IB and IF stigmas in 1997 to generate the first-generation back cross hybrids used in this study. Back crosses toward *I. brevicaulis* and backcrosses toward *I. fulva* will hereafter be referred to as BCIB and BCIF, respectively. F_1 hybrids were not included in this experiment because there was not enough rhizome material available. While F_1 hybrid fitness is important to the establishment of a hybrid zone in nature, we know from other experimental environments, F_1 hybrid fitness was very high (Johnston, Donovan, and Arnold, *unpublished data*). Additionally, once formed, hybrids tend to backcross toward parents rather than form F_2 and F_3 generations (Cruzan and Arnold 1994).

Plants in this study were clonally propagated from rhizome material produced in the greenhouse during the 1998-1999 growing season. Rhizome pieces 5-10 cm in length were excised from mature greenhouse-grown plants, trimmed of leaves and roots, and weighed prior to planting. Rhizome mass measurements were used as a covariate to account for initial size differences in statistical analyses. On November 30, 1999, 240 rhizomes were transplanted into 20 cm pots containing a 3: 1 sand: fritted clay (Turface, Buffalo Grove, IL, USA) mixture. Pots were arranged into 15 blocks on a greenhouse bench. All plants were fertilized throughout the experiment with Osmocote (Scotts Sierra, Marysville, OH, USA). For two weeks after planting, all plants were allowed to establish in full sun with watering to saturation once per day.

Experimental treatments

A schematic of the experimental setup is shown in Figure 3.1. The timing of our experiment was parallel to the natural growing season of these irises, December to April

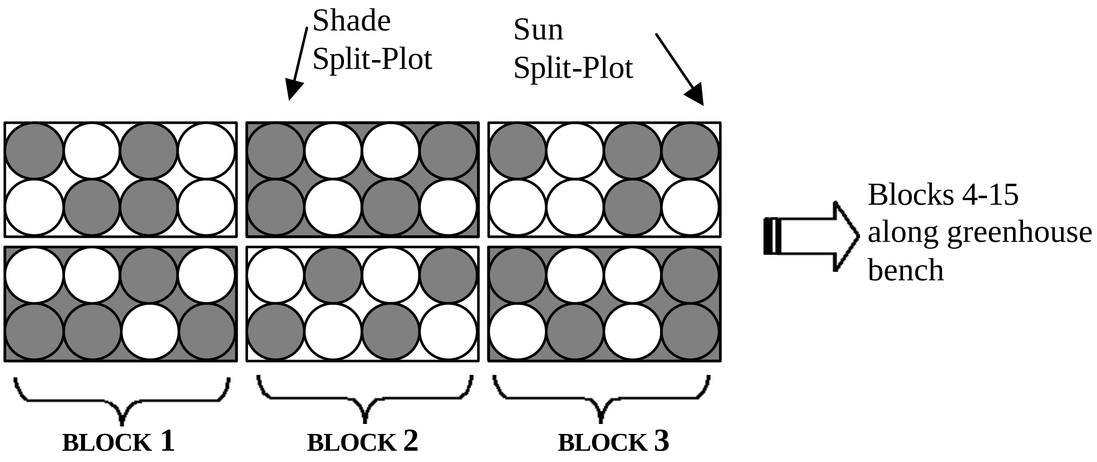

Figure 3.1. Experimental setup. Each circle represents a pot with an individual from one genotypic class. Shading of circles represents water treatments. Each rectangle containing eight pots represents the split plot structure for sun (open rectangle) and shade (shaded rectangle). Together, a pair of light split plots make a block. Each genotypic class*light*water combination was represented one time in each block. There were fifteen blocks in total.

(Arnold 1994). Three factors: light, water, and genotypic class (two species IB, IF, and two hybrid crosses BCIB, BCIF) were incorporated into a split plot experimental design. Shade and water treatments were initiated on Dec. 19, 1999. Each level of light treatment (sun or shade) was randomly assigned to one half of each block. Plants in the sun treatment were exposed to ambient light in the greenhouse. Plants in the shade treatment were covered by tents made from black cotton broadcloth (Jo-Ann Fabric, Hudson, OH), stretched on a PVC frame. Shade structures filtered out approximately 90% of ambient light, similar to the strongest shade treatment used in a previous study of shade tolerance in *I. fulva* and *I. hexagona* (Bennett 1989, Bennett and Grace 1990), and in the range of light expected to reach the floor of a fully leafed out a hardwood forest (Hutchinson and Matt 1977) . There were two levels of water treatment, damp (watered to saturation once per day) and dry (watered to saturation twice per week). Within light treatments, genotypic class and water treatment combinations were randomly assigned (Figure 3.1). Environmental treatment combinations were: sun/damp, sun/ dry, shade/damp, shade/dry. Plants were harvested on April 21-22, 2000.

Physiological measurements

Prior to harvest, photosynthetic rate (A), stomatal conductance (g_s), and internal CO_2 concentration (c_i) were measured on a random subset of plants with a LI-COR 6400 gas exchange system using a LI-6400-02 red light source (LI-COR, Lincoln, NE). During measurements, conditions around the leaf were as follows: 350 ppm CO_2, 26 ºC block temperature, and 25% humidity. Two light levels were used for gas exchange measurements. To gauge the efficiency of CO_2 assimilation under treatment conditions, plants in the sun treatment were measured at 1200 ìmol· m^{-2} · sec^{-1} and plants in the shade treatment were measured at 100 ìmol· m^{-2} · sec^{-1}, similar to the maximum light levels experienced in each light treatment on a sunny day.

Leaf area and number were measured over the course of the experiment to estimate growth rate. At treatment initiation, leaf area was non-destructively estimated by measuring length (to the nearest cm) and width at midpoint (to the nearest mm) of every leaf on each plant. Leaf area estimates were repeated at weeks 6, 12, and 18, and included all leaves greater than 5 cm long. Number of leaves, a measurement used in the field as a non-destructive estimate of plant size and vigor (Emms and Arnold 1997), was also recorded at each date.

Following harvest, leaf, rhizome and root tissues were separated, dried at 60°C to constant mass, and weighed separately. Biomass allocation was evaluated as the proportion of biomass in each tissue type: leaf mass ratio (leaf biomass / total biomass), rhizome mass ratio (rhizome biomass / total biomass), and root mass ratio (root biomass / total biomass). Harvest leaf area was measured with a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA). There was a strong correlation between non-destructive and leaf area meter measurements at harvest ($r^2 = 0.87$). Because leaves taper, estimated leaf areas were generally overestimates. Uncorrected leaf area estimates for each of the four measurement dates were used in the statistical analysis of growth. Leaf area ratio (LAR, leaf area / total biomass) and Specific Leaf Area (SLA, leaf area / leaf biomass) were calculated using leaf area measured at harvest to evaluate leaf allocation at the endpoint of the experiment.

Fitness Components

Louisiana irises are long-lived plants that reproduce both sexually and clonally. Therefore, lifetime fitness estimates must include both vegetative and sexual components (Wikberg 1995; Wikberg et al. 1994). When these plants experience dry conditions in the field, they rarely flower (Johnston, Arnold, and Donovan, *unpublished data*) so it was no surprise that none of the plants in this experiment flowered. Two vegetative components of fitness were measured as single-season estimators of fitness. At the beginning of the experiment, each rhizome had one growth point (above ground meristem). Each additional point of leaf growth initiation would became a new rhizome that was a viable vegetative ramet. Any point where leaf growth initiated and produced at least one leaf longer than 5 cm was counted as a new ramet. The number of ramets produced is a robust estimate of clonal reproductive effort (Wikberg 1995; Wikberg et al. 1994). In addition, we measured the most common character used to estimate clonal fitness, total standing biomass (e.g. Mendez and Obeso 1993; Solbrig 1981a; Winkler and Fischer 1999; Winkler et al. 1999) which represents the resources available to a plant for the next season's growth and sexual reproduction.

STATISTICAL ANALYSES

All data were analyzed as a split plot design using PROC MIXED in SAS (SAS 1989). Data were transformed as necessary to meet model assumptions (Sokal and Rohlf 1995). PROC MIXED uses maximum likelihood procedures to more precisely model random effects than more conventional sums of squares methods (Littell et al. 1996). Two effects (block and block*water) were modeled as random. Significance of the random effects is not tested directly by the procedure, but was evaluated with *ad hoc* Chisquare tests of maximum likelihood statistics (Littell et al. 1996). For all of our analyses presented here, block is not significant at the $p = 0.05$ level and block*light is significant in all analyses except for leaf weight ratio and rhizome weight ratio.

The response variables that were calculated per unit biomass or leaf area (gas exchange, biomass allocation, LAR, SLA) were analyzed using ANOVA in PROC MIXED. Total biomass and number of ramets produced were analyzed with an ANCOVA in PROC MIXED, including initial rhizome biomass as a covariate. Leaf area and leaf number estimates of growth were analyzed as repeated measures ANCOVAs with initial rhizome biomass as the covariate, and a first order autoregressive covariance matrix (Littell et al. 1996). The numbers presented in figures are LSMEANS and standard errors computed by SAS with untransformed data. By plotting LSMEANS, each variable can be shown with the effects of other terms in the model (such as the covariate) taken into account.

Transgressive trait expression was interpreted visually from graphs. In post hoc means comparisons, hybrid groups were never statistically distinct from both species for a given environmental treatment. We recognize that this method of interpretation allows us to comment only on the potential for transgressive trait expression of each hybrid class. However, since ultimately transgressive trait expression is important at the individual genotype level, shifts in the hybrid class mean are highly suggestive of transgressive expression by at least some genotypes within a hybrid group.

RESULTS

Physiological trait expression

Evidence of potential transgressive trait expression was found for several physiological characters. BCIB showed transgressive trait expression of leaf mass ration, rhizome mass ratio, and root mass ratio (Figure 3.2), and number of leaves (Figure 3.3). Number of leaves produced by BCIB was greater than parent species in sun treatments and less than parents in shade treatments, demonstrating transgressive expression of one trait in both a positive and negative direction. BCIF showed evidence of transgressive expression in SLA and rhizome mass ratio. BCIF expresses SLA in a way that is both positively and negatively transgressive in different environments, and the same is true for number of leaves in BCIB.

Environment-dependence of physiological traits

Biomass allocation to leaves and rhizomes was significantly affected by the main effects water and genotypic class and the interaction of water and light (Table 3.1). Leaf and rhizome mass ratios opposed each other across all environmental treatments, with leaf allocation greater in damp treatments and rhizome allocation greater in dry treatments (Figure 3.2). Root mass ratio was small, and was affected primarily by light treatments (Table 3.1). While there were no significant higher order interaction effects for root mass ratio (Table 3.1), the interaction between light and class is suggestive. IB in particular appeared to respond differently to the environmental treatments compared to other genotypic classes (Figure 3.2).

Photosynthetic tissue allocation variable SLA revealed differences in character expression among classes, especially in response to sun and shade (Table 3.1, Figure 3.2).

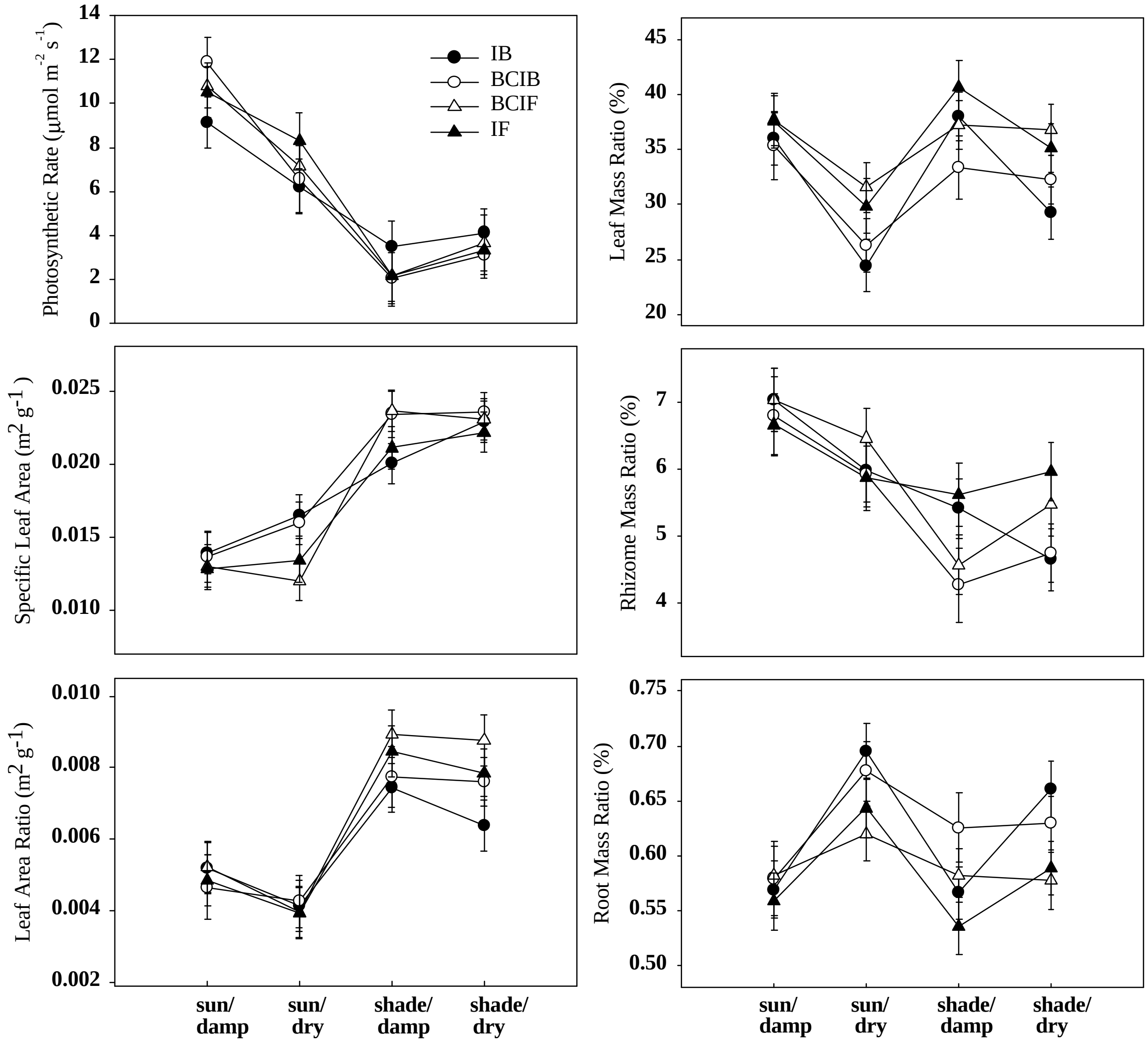

Figure 3.2. Physiological response of four Louisiana Iris genotypic classes (IB – *I. brevicaulis*, BCIB – back cross hybrids toward *I. brevicaulis*, BCIF – back cross hybrids toward *I. fulva*, IF – *I. fulva*) to four environmental treatments: sun/damp, sun/dry, shade/damp, shade/dry. Each response variable and its appropriate units are shown on the y-axis.

Table 3.1. Effects of water and light treatments on biomass allocation to leaves, rhizomes, and roots, and leaf tissue allocation in four genotypic classes of Louisiana *Iris* (IB, BCIB, BCIF, IF) in response to changes in water and light treatments. Results from SAS PROC MIXED.

			Allocation to tissue types						Photosynthetic allocation			
			Leaf weight ratio (%)		Rhizome weight ratio (%)		Root weight ratio (%)		LAR (m^2 g^{-1})		SLA (m^2 g^{-1})	
Source	ndf	ddf	F	p	F	p	F	p	F	p	F	p
light	1	14	3.67	**0.076**	1.33	0.268	12.6	**0.003**	31.4	**<0.001**	41.8	**<0.001**
water	1	146	26.7	**<0.001**	21.3	**<0.001**	1.48	0.227	5.64	**0.019**	3.45	0.065
genotypic class	3	146	3.73	**0.013**	3.12	**0.028**	1.87	0.137	1.11	0.345	1.86	0.138
light*water	1	146	4.58	**0.034**	4.11	**0.044**	6.31	**0.013**	1.25	0.266	0.19	0.666
light*class	3	146	0.18	0.910	0.33	0.804	2.34	0.076	1.78	0.153	3.31	**0.022**
class*water	3	146	1.9	0.133	2.61	0.054	1.27	0.287	0.22	0.882	2.01	0.115
light*class*water	3	146	0.35	0.787	0.32	0.810	0.85	0.478	0.12	0.946	0.28	0.842

Hybrid SLA responded to light and water conditions in ways that were similar to their closest parent species, except in shade/damp where both hybrids had greater SLA than both species (Figure 3.2). LAR was affected by both light and water, but there were not differences in the responses of genotypic classes.

Gas exchange parameters photosynthetic rate (A), conductance (g_s) and internal CO_2 (c_i) were all affected by light and water treatments (Table 3.2). Since measurements on sun and shade plants were taken at two different light levels, we expected lower photosynthetic rates in shade treatment plants. Photosynthetic rates were lower in sun/dry than in sun/damp, but slightly higher in shade/dry compared to shade/damp (Figure 3.2). A, g_s, and c_i for all genotypic classes responded to the environment in similar ways, providing no evidence of extreme hybrid trait expression.

Patterns of growth response varied among genotypic classes. Leaf area expansion and number of leaves produced were affected differently by environmental factors over the course of the experiment. Average leaf area was affected by initial rhizome weight, light, and an interaction between class and light (Table 3.3). Moreover, change in leaf number over time was affected by light, water, and genotypic class (Table 3.3). In the sun treatments, IB attained higher leaf area than any other genotypic class (LSMEAN ± SE =1127 ± 72 cm^2), and in shaded treatments, IF attains the highest leaf area (LSMEAN ± SE = 1119 + 70 cm^2). Leaf number responded differently to environmental treatments. Overall initial rhizome mass, genotypic class and time affected number of leaves. Over time, number of leaves produced was different among genotypic classes, and responded to water and water*light interaction (Table 3.3). Plants produced more leaves in sun than

Table 3.2. Statistical effects of light and water treatments on gas exchange parameters photosynthetic rate (A – µmol m^{-2} s^{-1}), stomatal conductance (g_s - mol s^{-2} m^{-1}) and internal CO2 concentration (c_i – ppm) on four genotypic classes of Louisiana *Iris* (IB, BCIB, BCIF, IF). Data were analyzed using ANOVA procedures in SAS PROC MIXED.

			A		g_s		c_i	
Source	ndf	ddf	F	p	F	p	F	p
light	1	14	94.6	**<0.001**	16.6	**0.001**	21.4	**<0.001**
water	1	67	4.19	**0.045**	56.3	**<0.001**	120.2	**<0.001**
light*water	1	67	14.8	**<0.001**	20.1	**<0.001**	10.5	**0.002**
genotypic class	3	67	0.05	0.984	0.77	0.513	0.4	0.751
light*class	3	67	1.28	0.290	0.21	0.888	0.83	0.480
water*class	3	67	0.3	0.822	2.35	0.080	0.14	0.937
light*water*class	3	67	0.35	0.789	0.24	0.866	1.37	0.259

Table 3.3. Summary of growth, analyzed as a repeated measures ANCOVA analyses in SAS PROC MIXED for number of leaves and leaf area produced by four genotypic classes of Louisiana *Iris* (IB, BCIB, BCIF, and IF).

			Leaf Area		Number of Leaves	
Source	ndf	ddf	F	p	F	p
Initial rhizome	1	631	14.0	**<0.001**	37.9	**<0.001**
light	1	14	1.2	0.294	6.64	**0.022**
water	1	631	3.05	0.081	1.49	0.223
genotypic class	3	631	3.98	**0.008**	2.3	0.076
light*water	1	631	1.93	0.165	2.89	0.090
light*class	3	631	2.07	0.103	5.45	**0.001**
class*water	3	631	0.39	0.759	0.11	0.952
light*class*water	3	631	0.66	0.579	1.63	0.181
time	3	631	793.7	**<0.001**	375.3	**<0.001**
light*time	3	631	0.81	0.488	5.1	**0.002**
water*time	3	631	16.9	**<0.001**	5.8	**<0.001**
class*time	9	631	6.21	**<0.001**	2.23	**0.019**
light*water*time	3	631	4.56	**0.004**	1.54	0.203
light*class*time	9	631	1.29	0.239	1.84	0.058
class*water*time	9	631	1.48	0.153	1.52	0.137
light*class*water*time	9	631	0.52	0.863	0.88	0.541

in shade treatments, and BCIB produced more leaves than the other genotypic classes in the sun (Figure 3.3).

Environment-dependence of hybrid fitness

Total biomass was affected by initial rhizome weight, main effects of light, class, and water, and an interaction of light and water (Table 3.4). There was no overall difference in biomass attained by genotypic classes (Table 3.4). All genotypic classes responded to light and water treatments in a similar way, as evidenced by no higher order interactions with class (Table 3.4). All genotypic classes were largest in the sun/damp treatment and smaller in the other, presumably more stressful treatments (Figure 3.4).

Number of ramets was affected by initial rhizome mass, but not by genotypic class, light, and water individually. However, genotypic classes produced different numbers of ramets in sun than in shade (Figure 3.4). Furthermore, a significant three-way interaction indicates that genotypic classes also produced different numbers of ramets in each light and water treatment combination (Table 3.4, Figure 3.4). The BCIB genotypic class produced the most variable number of ramets, with the most in sun/damp, and the least in damp/shade treatment (Figure 3.4). Both BCIB and BCIF produced fewer ramets than either parent in the shade/damp treatment (Figure 3.4). In two treatments, BCIF produced fewer ramets than either parent, and in no treatment did BCIF produce significantly more ramets than either parent (Figure 3.4). *Iris brevicaulis* had the largest number of ramets in the sun/dry and shade/damp treatments, while IF had the greatest number of ramets in the shade/dry treatment (Figure 3.4).

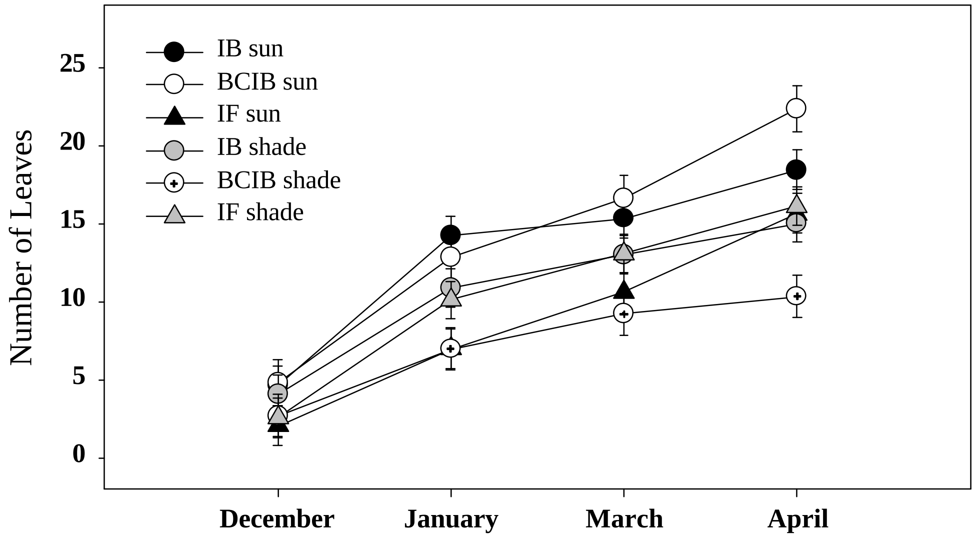

Figure 3.3. Number of leaves produced by three classes of Louisiana Iris (IB – *I. brevicaulis*, BCIB – back cross hybrids toward *I. brevicaulis,* IF – *I. fulva*) in sun and shade treatments, sampled at 6 week intervals over one growing season. Values presented are LSMEANS ± SE generated by repeated measures analysis in SAS.

Table 3.4. Effects of water and light treatments on fitness components total biomass and number of ramets produced by four genotypic classes of Louisiana *Iris* (IB, BCIB, BCIF, IF) in response to water and light treatments. Initial rhizome mass was included as a covariate in ANCOVA analysis. Results are from SAS PROC MIXED.

			Ramets		Biomass	
Source	ndf	ddf	F	p	F	p
Covariate	1	145	3.94	**0.049**	5.15	**0.025**
light	1	146	1.07	0.320	22.1	**<0.001**
water	1	146	0.01	0.933	16.7	**<0.001**
genotypic class	1	14	2.22	0.089	3.86	**0.011**
light*water	3	146	0.95	0.332	6.27	**0.013**
light*class	3	146	2.76	**0.044**	1.46	0.228
class*water	3	146	0.05	0.987	0.85	0.470
light*class*water	3	146	2.93	**0.036**	0.91	0.436

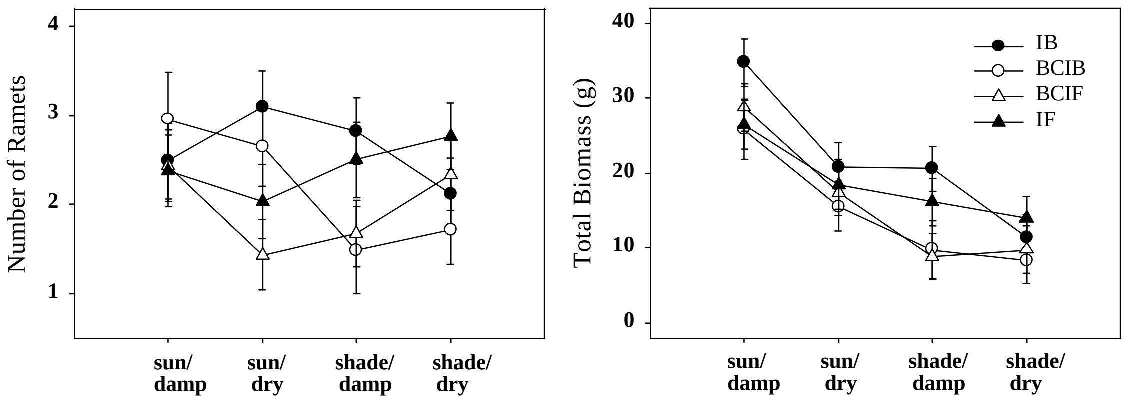

Figure 3.4. Fitness components total biomass and vegetative ramet production in four genotypic classes of Louisiana *Iris* across four environments. IB – *I. brevicaulis*, IF – *I. fulva*, BCIB and BCIF are first generation backcrossed hybrids toward IB and IF, respectively.

DISCUSSION

Physiological trait expression

In this study we found some evidence of transgressive trait expression by Louisiana *Iris* hybrids in every physiological character measured. Transgressive expression of any given trait was most often apparent in only one environment. It was difficult to predict the relative expression of hybrid traits from one environmental condition to the next. LAR was the only character for which positive transgressive trait expression occurred in multiple environmental treatments. Number of leaves was the one trait that shifted from positive to negative transgressive expression from one environmental treatment to another.

Environment-dependence of physiological traits

All genotypic classes experienced shifts in allocation patterns that were consistent with predicted “optimal” allocation patterns in response to light treatments (Lambers et al. 1998; Poorter and Lambers 1986a), but counter to the predicted response to water treatments (Larcher 1995). A greater proportion of resources were allocated to leaf tissue in the shade, but allocation to roots was lower in dry treatments than in damp. Since maximizing acquisition of the most limiting resource is presumably the optimal response to a stress, the reduced allocation to root tissue was a surprise. Root and rhizome allocation in the sun/dry treatment suggested that all genotypic classes might survive on stored resources during dry periods, rather than forage for water. Allocational responses to both light and water treatments were relatively consistent among all genotypic classes, suggesting that both species are physiologically similar, and that hybrids responded to the environment in similar ways to parent species.

We expected that inherent differences in shade tolerance between IB and IF would be reflected in gas exchange characters and leaf area expansion (Lambers et al. 1998; Larcher 1995). The two species had similar leaf area expansion rates: IB in sun and IF in shade. While growth rate (or leaf area expansion) is functionally linked to photosynthetic rate, SLA, and LAR (Poorter and Lambers 1986b), none of these underlying characters differed much among genotypic classes. Signals of hybrid novelty can be difficult to capture with instantaneous measurements such as gas exchange (Johnston et al. 2001a; Schwarzbach et al. 2001), but are more pronounced in integrative physiological characters, such as leaf area and biomass (Weber and D'Antonio 1999).

Environment-dependence of hybrid fitness

The two fitness components measured in this experiment, total biomass and ramet production did not respond to the environmental treatments in the same way (Figure 3.4). For total biomass, ranking of genotypic class performance did not change across environments. Overall, hybrids have a lower average fitness than parental species in the range of environments tested. Thus, our results from the biomass fitness component agree with some models of hybrid fitness that predict hybrids will always be unfit and have small evolutionary potential, regardless of their environment (Barton and Hewitt 1985; Harrison 1986). Because irises are clonal plants, there are several components to their fitness (Cheplick 1997; Wikberg et al. 1994). Vegetative and sexual reproduction are the most important components of clonal plant fitness (Wikberg 1995). Since no plants in our experiment flowered, all potential reproduction by these plants is clonal. Vegetative ramet production is extremely important in maintaining *Iris* populations in nature (Burke et al. 2000). Seedlings are rarely seen in the field, and germination is relatively infrequent (Johnston, Wesselingh, and Arnold *unpub. data*). Once an *Iris* rhizome becomes established, the likelihood of mortality for each ramet is small (Emms and Arnold 1997, but see Chapter 5). The results of the present study suggest that hybrids between *I. brevicaulis* and *I. fulva* species have lower vegetative reproductive fitness the parent species in the shade, but BCIB is the most fit genotypic class in the sun/damp environmental treatment. Taken together, these results suggest that Louisiana Iris hybrids in their natural habitat may have relatively high fitness in sunny areas, but are likely to be inferior to their parents in the shade. Thus, habitat that is heterogeneous for light at a fine scale, like that found along the bayous of southern Louisiana (Cruzan and Arnold 1993; Johnston et al. 2001b) may promote hybrid success.

Large standard errors within genotypic classes led us to choose to interpret transgressive trait expression graphically. When one or both hybrid genotypic class means fall outside the standard error of both parental species, this was considered evidence of potential transgressive segregation. We recognize that a more statistically rigorous means comparison would have been preferable. Our main objective was to show that hybrids have the potential for transgressive trait expression of physiological characters. Previous studies suggested that interactions between environmental factors were likely to be important in determining hybrid fitness (Johnston et al. 2001b), and we were willing to sacrifice some replication and statistical power to include more environmental treatments. We feel that the differences detected in the few genotypes from each hybrid class are very suggestive of the potential for hybrid novelty and transgressive trait expression in these irises. Future studies will include clonally replicated genotypes or increased sample sizes that allow for more robust evaluations of transgressive segregation.

Conclusions

To summarize, we find some evidence of transgressive trait expression in physiological characters in hybrids between *I. brevicaulis* and *I. fulva*. Transgressive expression was generally only found in one or two of the four environmental treatment combinations, indicating strong environment dependence of the phenomenon. Much of the time, physiological characters of all genotypic classes responded to the environment in the same way, indicating that all are limited by dry, shaded conditions. Fitness components biomass and ramet production gave contradictory accounts of relative hybrid fitness and its dependence on environmental conditions. Conservatively, we conclude that hybrids have slightly lower fitness than parents in dry areas, and that can be partially overcome in sunny conditions.

ACKNOWLEDGEMENTS

The authors wish to thank A. Tull and M. Boyd for assistance in caring for our plants at the UGA Botany Greenhouse during the course of this experiment. We appreciate comments on earlier versions of this manuscript from A. Bouck, S. Cornman, J. Espeleta, E. Kentner, C. Richards, D. Rosenthal, K. Snyder, J. Hamrick, S. Chang, C. Peterson, and R. Sharitz. We would also like to thank G. Guest, V. Jin, S. Franks, J. Cruse, A. Salmore, and R. Klaper for help with the harvest. This research was supported by an American Iris Society scholarship to JAJ and National Science Foundation grant DEB-0074159 to MLA.

LITERATURE CITED

Anderson, E. 1949. Introgressive hybridization. Wiley & Sons, New York.

Arnold, M. L. 1992. Natural Hybridization as an Evolutionary Process. Annual Review of Ecology and Systematics 23:237-261.

Arnold, M. L. 1993. *Iris-Nelsonii* (Iridaceae) - Origin and Genetic Composition of a Homoploid Hybrid Species. American Journal of Botany 80:577-583.

Arnold, M. L. 1994. Natural Hybridization and Louisiana Irises. Bioscience 44:141-147.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Arnold, M. L., E. K. Kentner, J. A. Johnston, S. Cornman, and A. C. Bouck. 2001. Natural hybridisation and fitness. Taxon 50:93-104.

Arntz, A. M., E. H. DeLucia, and N. Jordan. 1998. Contribution of photosynthetic rate to growth and reproduction in Amaranthus hybridus. Oecologia 117:323-330.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16:113-148.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the Fitness of Hybrids. Annual Review of Genetics 35:31-52.

Burke, J. M., M. R. Bulger, R. A. Wesselingh, and M. L. Arnold. 2000. Frequency and spatial patterning of clonal reproduction in Louisianan *Iris* hybrid populations. Evolution 54:137-144.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998a. Hybrid fitness in the Louisiana irises: Analysis of parental and F1 performance. Evolution 52:37-43.

Burke, J. M., T. J. Voss, and M. L. Arnold. 1998b. Genetic interactions and natural selection in Louisiana iris hybrids. Evolution 52:1304-1310.

Cheplick, G. P. 1997. Responses to severe competitive stress in a clonal plant: differences between genotypes. OIKOS 79:581-591.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and Genetic Associations in an Iris Hybrid Zone. Evolution 47:1432-1445.

Cruzan, M. B., and M. L. Arnold. 1994. Assortative mating and natural selection in an *Iris* hybrid zone. Evolution 48:1946-1958.

Emms, S. K., and M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness: Transplant experiments with Louisiana irises. Evolution 51:1112-1119.

Emms, S. K., S. A. Hodges, and M. L. Arnold. 1996. Pollen-tube competition, siring success, and consistent asymmetric hybridization in Louisiana irises. Evolution 50:2201-2206.

Ferguson, D., and T. Sang. 2001. Speciation through homoploid hybridization between allotetraploids in peonies (Paeonia). Proceedings of the National Academy of Sciences of the United States of America 98:3915-3919.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, San Diego, CA, USA.

Harrison, R. G. 1986. Pattern and Process in a Narrow Hybrid Zone. Heredity 56:337-349.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution 53:866-873.

Hewitt, G. M. 1988. Hybrid Zones - Natural Laboratories for Evolutionary Studies. Trends in Ecology & Evolution 3:158-167.

Hodges, S. A., J. M. Burke, and M. L. Arnold. 1996. Natural formation of iris hybrids: Experimental evidence on the establishment of hybrid zones. Evolution 50:2504-2509.

Huenneke, L. F., and R. R. Sharitz. 1986. Microsite Abundance and Distrbiution of Woody Seedlings in a South Carolina Cypress-Tupelo Swamp. American Midland Naturalist 115:328-335.

Hutchinson, B. A., and D. R. Matt. 1977. The distribution of solar radiation within a deciduous forest. Ecological Monographs 47:185-207.

Johnston, J. A., D. J. Grise, L. A. Donovan, and M. L. Arnold. 2001a. Environment-dependent performance and fitness of *Iris brevicaulis*, *I. fulva* (Iridaceae), and hybrids. American Journal of Botany 88:933-938.

Johnston, J. A., R. A. Wesselingh, A. C. Bouck, L. A. Donovan, and M. L. Arnold. 2001b. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana Iris hybrid population. Molecular Ecology 10:673-681.

Lambers, H., F. S. Chapin, and T. L. Pons. 1998. Plant Physiological Ecology. Springer-Verlag, New York, NY, USA.

Larcher, W. 1995. Physiological Plant Ecology. Springer-Verlag, Berlin.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315-&.

Littell, R. C., G. A. Milliiken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute, Cary, NC, USA.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Mendez, M., and J. R. Obeso. 1993. Size-Dependent Reproductive and Vegetative Allocation in Arum- Italicum (Araceae). Canadian Journal of Botany-Revue Canadienne De Botanique 71:309-314.

Osmond, C. B., M. P. Austin, J. A. Berry, W. D. Billings, J. S. Boyer, J. W. H. Dacey, P. S. Nobel, S. D. Smith, and W. E. Winner. 1987. Stress Physiology and the Distribution of Plants. Bioscience 37:38-48.

Poorter, H., and H. Lambers. 1986a. Growth and Competitive Ability of a Highly Plastic and a Marginally Plastic Genotype of *Plantago major* in a Fluctuating Environment. Physiologia Plantarum 67:217-222.

Poorter, H., and H. Lambers. 1986b. Growth and Competitive Ability of a Highly Plastic and a Marginally Plastic Genotype of Plantago-Major in a Fluctuating Environment. Physiologia Plantarum 67:217-222.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg, L. H., and N. C. Ellstrand. 1993. What Can Molecular and Morphological Markers Tell Us About Plant Hybridization. Critical Reviews in Plant Sciences 12:213-241.

Rieseberg, L. H., C. Vanfossen, and A. M. Desrochers. 1995. Hybrid Speciation Accompanied by Genomic Reorganization in Wild Sunflowers. Nature 375:313-316.

Riley, H. P. 1938. A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. American Journal of Botany 25:649-725.

Rosenthal, D. M., A. E. Schwarzbach, L. A. Donovan, O. Raymond, and L. H. Rieseberg. 2002. Phenotypic differentiation between three anient hybrid taxa and their parental species. International Journal of Plant Sciences 163:387-398.

Santiago, L. S., T. S. Lau, P. J. Melcher, O. C. Steele, and G. Goldstein. 2000. Morphological and physiological responses of Hawaiian Hibiscus tiliaceus populations to light and salinity. International Journal of Plant Sciences 161:99-106.

SAS. 1989. SAS/STAT user's guide, version 6. SAS Institute, Cary, NC.

Schwarzbach, A. E., L. A. Donovan, and L. H. Rieseberg. 2001. Transgressive character expression in a hybrid sunflower species. American Journal of Botany 88:270-277.

Snow, A. A., and S. W. Vince. 1984. Plant Zonation in an Alaskan Salt-Marsh .2. An Experimental- Study of the Role of Edaphic Conditions. Journal of Ecology 72:669-684.

Sokal, R. R., and J. F. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., New York.

Solbrig, O. T. 1981a. Studies on the Population Biology of the Genus *Viola* .2. The Effect of Plant Size on Fitness in *Viola sororia*. Evolution 35:1080-1093.

Solbrig, O. T. 1981b. Studies on the Population Biology of the Genus Viola .2. The Effect of Plant Size on Fitness in Viola-Sororia. Evolution 35:1080-1093.

Tilman, D. 1997. Mechanisms of Plant Competition *in* M. J. Crawley, ed. Plant Ecology. Blackwell Science, Oxford, U.K.

Viosca, P. 1935. The irises of Southeastern Louisiana: a taxanomic and ecological interpretation. Bulletin of the American Iris Society 57:3-56.

Walter, H. 1979. Vegetation of the earth in realtion to climate and the eco-physiological conditions. Springer-Verlag, New York, USA.

Weber, E., and C. M. D'Antonio. 1999. Phenotypic plasticity in hybridizing Carpobrotus spp. (Aizoaceae) from coastal California and its role in plant invasion. Canadian Journal of Botany-Revue Canadienne De Botanique 77:1411-1418.

Wikberg, S. 1995. Fitness in Clonal Plants. Oikos 72:293-297.

Wikberg, S., B. M. Svensson, and B. A. Carlsson. 1994. Fitness, Population-Growth Rate and Flowering in Carex- Bigelowii, a Clonal Sedge. Oikos 70:57-64.

Winkler, E., and M. Fischer. 1999. Two fitness measures for clonal plants and the importance of spatial aspects. Plant Ecology 141:191-199.

Winkler, E., M. Fischer, and B. Schmid. 1999. Modelling the competitiveness of clonal plants by complementary analytical and simulation approaches. Oikos 85:217-233.

CHAPTER 4

EVIDENCE OF NOVEL PHENOTYPES AMONG EARLY GENERATION HYBRIDS OF TWO LOUISIANA IRIS SPECIES IN TWO WATER TREATMENTS[1]

---

[1] J. A. Johnston, L. A. Donovan, and M. L. Arnold. To be submitted to *Evolution*.

ABSTRACT

Recent studies of hybridization assert that novel variation can be generated during massive genetic recombination events. Novel extreme phenotypes, though rare, may have the potential to contribute to ecological divergence of hybrid lineages. Extreme traits may only be expressed by certain genotype by environment interactions. Most studies of environment-dependent hybrid phenotypes have reported group averages for experimental or wild collected hybrid crosses. Louisiana irises, a naturally hybridizing species group, can be propagated from rhizomes and a single genotype replicated in multiple environments. Here we analyze physiological traits and fitness components of hybrids between *Iris brevicaulis* and *Iris fulva* as genotypic classes and as individual genotypes. Although extreme or novel phenotypes are likely to be rare, we find evidence of such at both the genotypic class-level and individual genotype-level. As a class, F_1 hybrids had significantly larger specific leaf area, root weight ratio, growth rate, total biomass, and number of vegetative ramets than both parental species. Additionally, both backcross hybrid classes possessed traits that were significantly extreme in a positive and negative fashion. Genotype-level analysis exposed one F_1 genotype with an extremely different pattern of root allocation, and another with very high specific leaf area that was not apparent at the class-level. Growth and total biomass at the individual genotype-level revealed three genotypes that were significantly larger than parental genotypes. Here we have rigorously demonstrated that early generation hybrid classes contain rare genotypes with environment-dependent expression of extreme phenotypic traits. Hybrid fitness was equal or superior to both parental species in many cases, supporting the prediction that novel hybrid traits may be important to plant evolutionary ecology.

INTRODUCTION

The role of hybridization as a creative force in evolution has recently gained new attention by evolutionary biologists. Contrary to the opinion that was common among architects of the modern synthesis (Barton and Hewitt 1985; Dobzhansky 1940; Mayr 1942), recent studies suggest that hybrids are relatively common in nature (Arnold et al. 2001; Rieseberg 1997; Rieseberg and Ellstrand 1993) and can contain novel genetic variation (Anderson and Stebbins 1954; Arnold 1992; Burke and Arnold 2001; Lewontin and Birch 1966; Rieseberg 1997; Rieseberg et al. 1999; Schwarzbach et al. 2001). The traditional expectation of intermediacy in hybrid phenotypes is not unsupported by empirical studies (Campbell and Waser 2001; Hatfield and Schluter 1999; Riley 1938). However, reports of unique hybrid phenotypes have accumulated, (reviews in Arnold 1992; Ellstrand and Schierenbeck 2000) prompting theoreticians to predict that rare but extreme hybrid phenotypes may be important in plant evolution (Arnold 1997; Rieseberg et al. 1999).

There are several ways extreme hybrid phenotypes could be generated (Devicente and Tanksley 1993). Rieseberg et al. (1999) argue that transgressive segregation is the most likely underlying genetic cause of extreme expression in quantitative traits. When parents possess alleles that contribute to a trait in an additive fashion, the sum of alleles in recombinant generations may lead to a broader range of phenotypes than is found in either parent species (de Vicente and Tanksley 1993; Rieseberg et al. 1999). Extreme or transgressive expression of one or more physiological or ecological traits would almost certainly affect hybrid fitness (Rieseberg et al. 1999).

Several theoretical frameworks have been proposed for studying hybrid zone evolution (reviews in Arnold 1997; Hewitt 1988). The Hybrid Novelty model (Arnold 1997) predicts that rare individuals in hybrid swarms can have higher fitness than either parental species by possessing unique traits or combinations of traits that are not present in either parental species. The fitness advantage enjoyed by novel hybrid genotypes is predicted to be environment-dependent (Arnold 1997Barton 2001; Rieseberg et al. 1999). Thus, if a hybrid genotype possesses a trait that permits the tolerance of an extreme habitat (e.g. flooded areas), the traits that confer that tolerance may not increase, or even decrease fitness other environments (Ernst 1990). If a new hybrid lineage establishes in novel habitat, it could lead to the range expansion of one parental species via introgression of ecological characters (Lewontin and Birch 1966), ecological divergence of the hybrid line (Grant and Grant 1996), or speciation (Arnold 1993). The alternative models of hybrid zone evolution, the Tension Zone (Barton and Hewitt 1985), Bounded Hybrid Superiority (Moore 1977) and Mosaic models (Harrison 1986; Howard 1986) predict that hybrids are likely to have low fitness due to genetic complications, and have little or no evolutionary impact.

Hybridization has already left its mark on the ecology of the Louisiana Iris species complex (summary in Arnold 2000). Louisiana irises are a small group of long-lived, rhizomatous, herbaceous perennial species whose distributions overlap in southern Louisiana (Arnold 1994; Viosca 1935). Two of the species, *Iris brevicaulis* and *I. fulva*, are distinct floristically, yet similar enough in their ecological distributions to co-occur (Cruzan and Arnold 1993 Johnston et al. 2001b) and hybridize fairly often (although F_1s are rare in nature). Both species occur along the edges of bayous (linear, freshwater, tidally influenced wetlands), but in different microhabitats (Cruzan and Arnold 1993). *Iris brevicaulis* grows in areas that are slightly drier and sunnier, while *I. fulva* thrives in shadier, wetter habitat (Johnston et al. 2001a). *Iris* species can colonize diverse microhabitat patches by seed or vegetative rhizome (Burke et al. 2000a; Cruzan and Arnold 1993; Johnston et al. 2001b). Sexual reproductive fitness of *I. brevicaulis* and *I. fulva* hybrids varies among genotypes, and on average is often equal or better to parent species (Burke et al. 1998b; Wesselingh and Arnold 2000). Seedling germination is rarely observed in the field (Johnston, Wesselingh, and Arnold, *unpublished data*), suggesting that traits contributing to survival and production of vegetative ramets are important to fitness of Louisiana *Iris* genotypes. Thus, transgressive segregation that affects traits related to growth, survival, and production of vegetative ramets in new environments, will likely affect fitness of hybrids.

In this study, we have measured physiological and fitness related characters of *I. brevicaulis, I. fulva*, and their hybrids to assess the potential for trait expression and hybrid novelty to be present in early generation hybrids. Specifically, we were interested in two questions at two levels. First, is there transgressive trait expression in early generation hybrids between *I. brevicaulis* and *I. fulva*? Second, does relative hybrid fitness change across environments? These two questions were posed at the genotypic class-level and the individual genotype-level. Finally, we will examine our findings at both levels, and compare the information that were gleaned from each.

METHODS

Plant Material

Two species, *Iris brevicaulis* and *I. fulva*, and three classes of their early generation hybrids were used in this experiment. Rhizomes from both species were collected in the field in 1994 (*I. brevicaulis* - Assumption Parish, *I. fulva* – Terrebonne Parish, both in Louisiana, U.S.A.). Hereafter the two species will be referred to as IB and IF respectively. Rhizomes were transplanted into potting soil and allowed to grow under well-watered, fertilized conditions in the greenhouse. Following transplantation to the greenhouse, every September plants were excavated from their pots and rhizome material divided. One rhizome was transplanted into a fresh pot with new potting soil and returned to the greenhouse. In early spring of 1995, crosses were initiated between IB and IF to produce F_1 hybrids. Seeds from these crosses were planted in summer 1995, and in spring of 1996, pollen from F_1 flowers was placed back onto IB and IF stigmas to produce first generation backcrosses toward IB and IF. Hybrid genotypic classes will be referred to as F_1, BCIB (backcross toward IB), and BCIF (backcross toward IF). By fall 1998, all genotypic classes used in this experiment had been grown under common greenhouse conditions for at least two years.

On September 4-5, 1998, individual plants that had produced more than six rhizomes during the 1997-1998 growing season were selected for potential use in the experiment. All available rhizomes were divided, trimmed of leaves and roots, rinsed and dried, then weighed (for use as covariate in statistical analyses). Each rhizome was planted in its own 18 cm diameter pot in pine bark potting mix. Pots were placed onto a greenhouse bench and allowed to establish in full sun, under well-watered conditions for

6 weeks. On October 28, five genotypes were selected and random from those that had six successful clones survive transplantation. All ramets used in the experiment had at least one leaf longer than 10 cm. Genotypes were randomly assigned to one of two water treatments.

Flood treatments

Two levels of water treatment, wet and flood were used in the experiment. In the wet treatment, plants were placed into standing water up to the soil surface, while flooded plants were in standing water 10 cm above soil surface. The experimental design was a split plot consisting of fifteen pairs of plastic tubs, one each assigned to wet and flood (Figure 1). Each tub held 5 pots, one with an individual of each genotypic class. Tubs were kept full by top watering with a hose 4 times each week. Holes drilled in the side of the tubs ensured that correct water level was maintained. To maintain a low dissolved oxygen concentration in the flood treatment tubs, nitrogen gas (N_2) was bubbled through the water in each tub for 20 minutes following watering. Throughout the experiment, relative humidity, ambient light level, and air and water temperature were monitored.

Response variables

Several physiological characters were measured on each plant during the course of the experiment. Leaf area was measured at five-week intervals throughout the experiment as a non-destructive estimate of growth rate (10/28 – treatment initiation, 12/02, 01/06, 02/10, 03/17. 04/21). All leaves that were at least 5 cm long were measured. Leaf area was estimated by measuring the length (to closest cm) and width at midpoint of leaf (to nearest mm) and calculating the area of the resulting rectangle. A subset of leaf areas were measured destructively using the LI-3000 leaf area meter (Li-

Cor, Lincoln, NE) at the end of the experiment. Regression analysis in Sigma Plot 2000 (SPSS Science, Chicago, IL, USA) indicates a strong correlation between our leaf area estimates and actual leaf area ($r^2 = 0.89 – 0.92$).

On April 21 and 22, 1999, all plants were harvested. Leaves and flower stalks were cut at rhizome surface, and roots and rhizomes were washed away from soil. Leaves, rhizomes, roots, and flower stalks were separated and dried at 60 °C for 48 hours. Biomass proportions were calculated from final dry weights: leaf weight ratio = leaf mass / total; rhizome weight ratio = rhizome mass / total; root weight ratio = root mass / total). To evaluate allocation within photosynthetic tissues, leaf area ratio (leaf area / total biomass) and specific leaf area (leaf area / leaf biomass) were calculated from estimated leaf areas at harvest. Two types of flood-specific responses were also measured upon harvesting, shallow root allocation and root and rhizome tissue density. To measure a shift in root allocation into upper, more oxygenated soil layers, pots were sliced in half horizontally prior to washing roots from soil. Roots in the top and bottom halves of the pot were dried and weighed separately, and shallow root ratio (top roots/total roots) was calculated. A small amount of fresh root and rhizome tissue was set-aside during harvest for density measurements. Low density would have indicated the presence of aerenchyma, which can keep submerged tissue supplied with oxygen. All roots and a subset of rhizomes were measured for tissue density with a Eureka apparatus and the water displacement method of Curran et al. 1996). Tissue subsamples were returned to their parent samples prior to drying.

Three traits measured during the experiment were considered to be important components of fitness, total biomass, number of rhizomes, and number of flowers. There are several components of lifetime fitness in a clonal plant (Gardner and Mangel 1999; Wikberg 1995). I the absence of any reproductive estimate, total biomass is usually considered the best estimate of plant fitness (Harper 1977; Wikberg et al. 1994). Number of ramets serves as an estimator of vegetative reproduction (Wikberg 1995). Each rhizome that was initiated during the growing season was counted as a new ramet. Flowers were recorded as they appeared (January through April) as an estimate of sexual reproductive potential.

Statistical Analyses

The experiment was designed as a split-plot. There were two levels of water (wet and flood), and five levels of genotypic class (IB, IF, F_1, BCIB, BCIF). The 15 pairs of tubs, each containing one plant from each genotypic class in flood and wet treatments, were each a replicate of the experimental unit and treated as blocks (refer to Figure 4.1). All analyses were performed with SAS statistical software (SAS Institute, Cary, NC, U.S.A.) using PROC MIXED (SAS 1989). PROC MIXED was chosen over more traditional sums of squares procedures (i.e. PROC GLM) because it explicitly models random effects (Littell et al. 1996). For our analyses, block and block*water were modeled as random.

Class-level analyses were conducted with main effects water, class, and the random effect block. Traits that were ratios, and therefore scaled to plant size (leaf weight ratio, rhizome weight ratio, root weight ratio, specific leaf area, leaf area ratio, top root ratio, root and rhizome density) were analyzed with ANOVAs. Traits that were measures of absolute plant size (total biomass, ramets, and flowers) were computed as ANCOVAs, with initial rhizome mass as the covariate. Growth was analyzed as a split

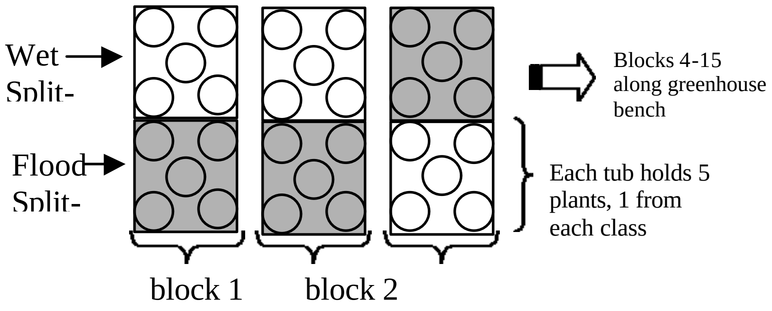

Figure 4.1. Schematic of experimental set-up. Each circle represents one pot with a plant in it, and each square containing 5 circles is one plastic tub. A pair of tubs, one open (wet) and one shaded (flood) comprise one experimental replicate or block. Fifteen blocks were arranged in a line down a greenhouse bench for this experiment.

plot repeated measures ANCOVA, with a first order autoregressive covariance structure and initial rhizome as a covariate. Means comparisons among classes were made with contrasts. Hybrid genotypic classes were considered transgressive when they were extreme relative to IB and IF, and were significantly different than at least one parental species in means comparisons. Proportional data were arcsine square root transformed before they were analyzed. Data that did not meet the normality requirement of ANOVA were square root transformed.

Each of the five classes in this study was composed of five genotypes. Each genotype was replicated three times in each water treatment. This replication allowed analysis and means comparisons among individual genotypes. Analysis at the genotype-level used the same data and analysis structure as at the class level, with slight modification. For genotype-level analysis, genotype, water, and block (as random) were the main effects in the statistical models. Means comparisons were made with contrasts among genotypes. Hybrid genotypes were considered transgressive when they were extreme relative to all IB and IF genotypes, and were significantly different from at least eight of the ten parental genotypes in means comparisons. Rhizome tissue density data were not analyzed at the genotype-level, because they were measured on a subset of plants.

RESULTS

Class-level analyses

Several physiological characters were significantly different among genotypic classes. Many of them exhibited transgressive trait expression as a class. There was a significant main effect of genotype on all biomass allocation patterns (Table 4.1). The F_1

Table 4.1. Biomass allocation in Louisiana irises at the genotypic class level. Five genotypic classes (IB, BCIB, F_1, BCIF, and IF) were analyzed for response to watering treatment (wet and flooded) using ANOVA analysis in SAS PROC MIXED. Effects that are significant at $p< 0.05$ are in bold.

			Leaf Area Ratio (m^2/g)		Specific Leaf Area (m^2/g)		Leaf Mass Proportion (g/g)		Rhizome Mass Prop. (g/g)		Root Mass Proportion (g/g)	
Source	ndf	ddf	F	p	F	p	F	p	F	p	F	p
Class	4	112	5.48	**<0.001**	4.72	**0.002**	6.51	**<0.001**	3.49	**0.010**	8.34	**<0.001**
Water	1	14	0.01	0.940	0.26	0.619	0.12	0.738	1.18	0.296	21.41	**<0.001**
Class*water	4	112	1.2	0.314	0.62	0.652	1.81	0.131	2.49	**0.048**	0.83	0.508

genotypic class was positive transgressive for rhizome weight ratio, root weight ratio, and specific leaf area (Figure 4.2). The performance of BCIB was more variable, being negative transgressive for leaf weight ratio and leaf area ratio, but positive transgressive for rhizome weight ratio and root weight ratio. BCIF hybrids were similarly split: negative transgressive for rhizome weight ratio, leaf area ratio, and positive transgressive for root weight ratio. Among flood response characters, top root ratio and root density were both significantly different among genotypic classes (Table 4.2). All hybrid classes were positively transgressive for top root ratio in either wet or flood treatments (Figure 4.2). There was no significant class-level transgressive expression of root or rhizome density. Rhizome density did not differ among genotypic classes (Table 4.2).

Growth rate varied significantly among classes (Table 4.3 – class*time). Two hybrid classes, F_1 and BCIF grew faster than IB and IF, the F_1 exhibiting positively transgressive growth in the flood water treatment (Figure 4.3).

Fitness components were significantly different among genotypic classes (Table 4.4). Patterns were similar for total biomass and number of ramets (Figure 4.4). All hybrid classes were positively transgressive for total biomass. The F_1 hybrid class was also positive transgressive for number of ramets, while BCIB as a class was negative transgressive (Figure 4.4). Sexual reproductive fitness patterns were different from size measurements (Table 4.4). IB produced very few flowers on average, while IF produced several per plant. All hybrid classes flowered at intermediate or IF-like levels (Figure 4.4).

Several instances of transgressive trait expression were only significant in one water treatment, but overall, class by water interactions were not significant. The only

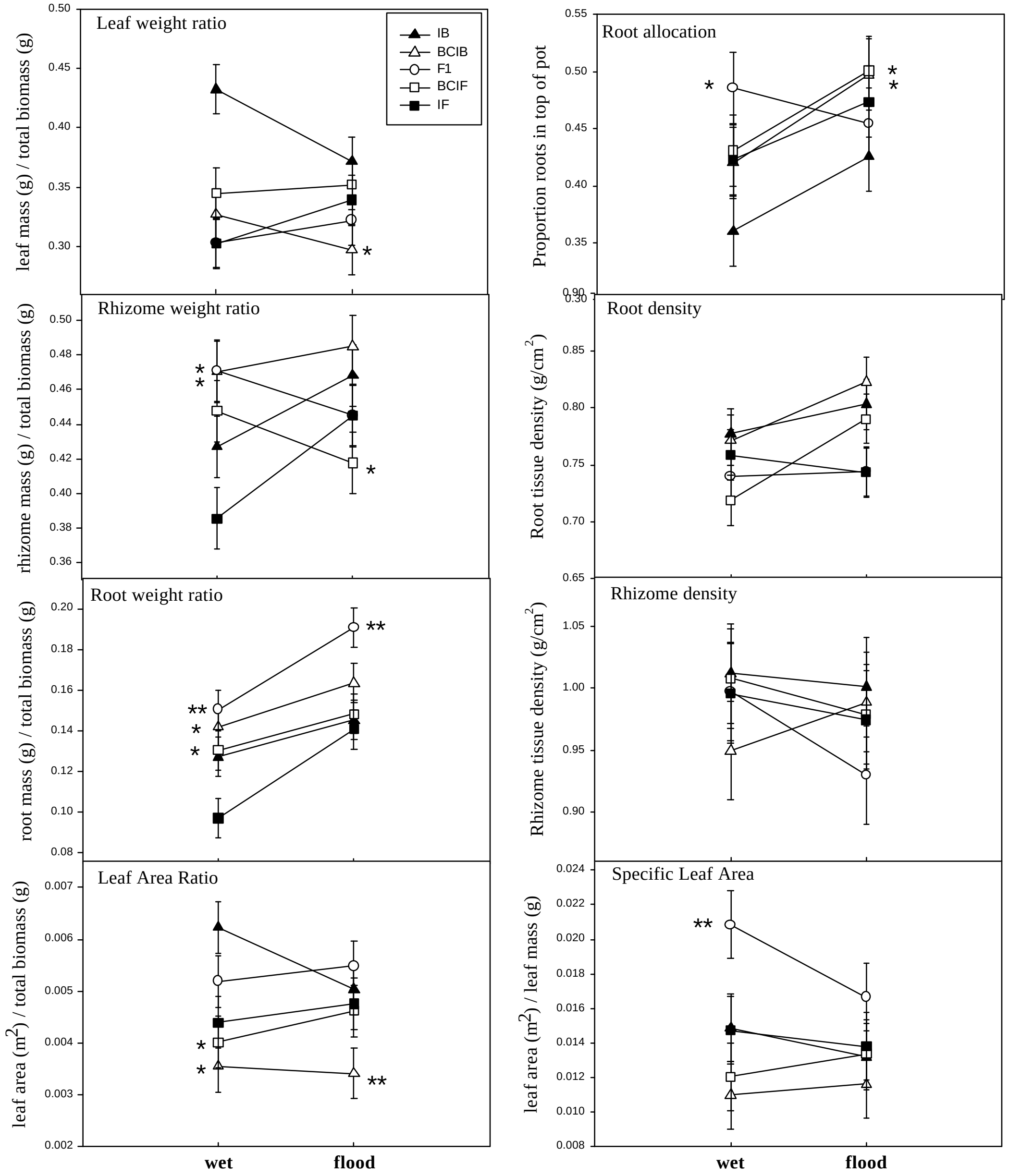

Figure 4.2. Genotypic class averages of allocation patterns and flood tolerance traits (LSMEANS from SAS ± SE). Hybrid genotypic classes are shown in open symbols, and those that are transgressive are indicated with asterisks. “**” indicates a hybrid class that is extreme and significantly different from both parental species in a means comparison ($p<0.05$), “*” indicates a hybrid class that is extreme relative to both parent species but only significantly different from one ($p<0.05$).

Table 4.2. Flood tolerance trait analysis for Louisiana irises at the genotypic class level. There were five levels of genotypic class (IB, BCIB, F_1, BCIF, and IF), and two levels of water (wet, flooded). Results are from PROC MIXED in SAS. Effects that are significant at $p< 0.05$ are in bold.

			Root allocation (g/g)		Root density (g/ml)				Rhizome density (g/ml)	
Source	ndf	ddf	F	p	F	p	ndf	ddf	F	p
Class	4	112	2.57	**0.042**	2.61	**0.039**	4	32	0.41	0.801
Water	1	14	9.74	**0.008**	3.94	0.067	1	4	0.47	0.531
Class*water	4	112	0.38	0.823	1.27	0.285	4	32	0.50	0.739

Table 4.3. Non-destructive growth rate analysis (from leaf area measurements) for Louisiana irises at the genotypic class level. Genotypic classes are IB, BCIB, F_1, BCIF, and IF, and initial rhizome biomass is the covariate. Plants were subjected to wet and flood watering treatments, and measured six times.

			Leaf area (cm^2)	
Source	ndf	ddf	F	p
covariate	1	811	261	**<0.001**
class	4	811	9.49	**<0.001**
water	1	14	6.08	**0.027**
class*water	4	811	0.54	0.703
time	5	811	453	**<0.001**
class*time	20	811	3.19	**<0.001**
water*time	5	811	3.38	**0.005**
class*water*time	20	811	0.76	0.761

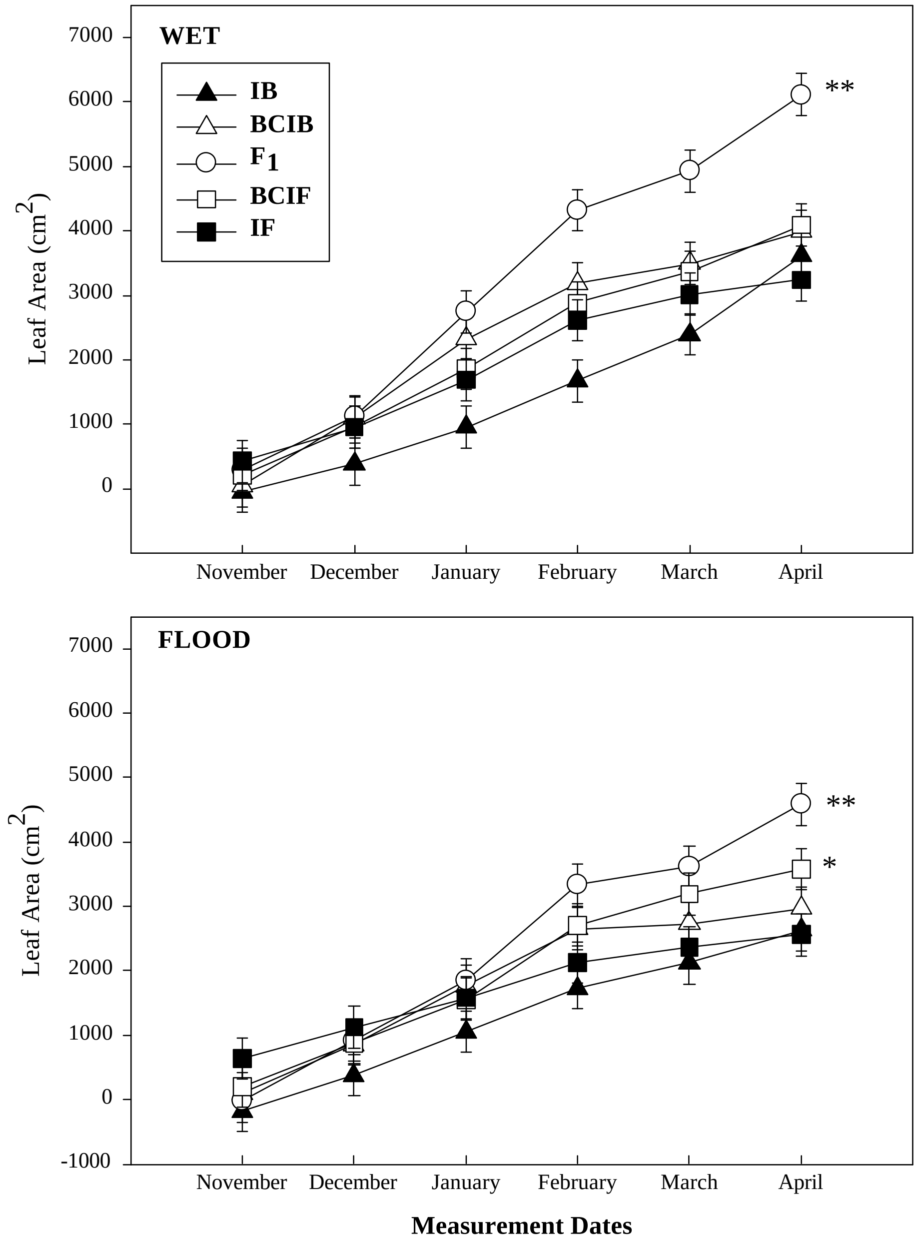

Figure 4.3. Genotypic class level non-destructive growth (leaf area) measurements over the course of the experiment (LSMEANS from SAS ± SE). Transgressive genotypes are indicated with asterisks. "**" indicates an extreme hybrid class that is significantly different from the mean of both parental species, and "*" indicates an extreme hybrid class that is significantly different from the mean of one species.

Table 4.4. Fitness component analysis for Louisiana irises at the genotypic class level. The five genotypic classes are (IB, BCIB, F_1, BCIF, and IF) and water treatments were wet and flooded. Effects significant at $p < 0.05$ are in bold.

			Total biomass (g)		Ramets (#)		Flowers (#)	
Source	ndf	ddf	F	p	F	p	F	p
Covariate	1	111	38.7	**<0.001**	45.6	**<0.001**	10.7	**0.001**
Class	4	111	10.3	**<0.001**	5.72	**<0.001**	8.94	**<0.001**
Water	1	14	9.53	**0.008**	8.38	**0.012**	4.07	0.063
Class*water	4	111	0.49	0.746	0.51	0.729	0.74	0.567

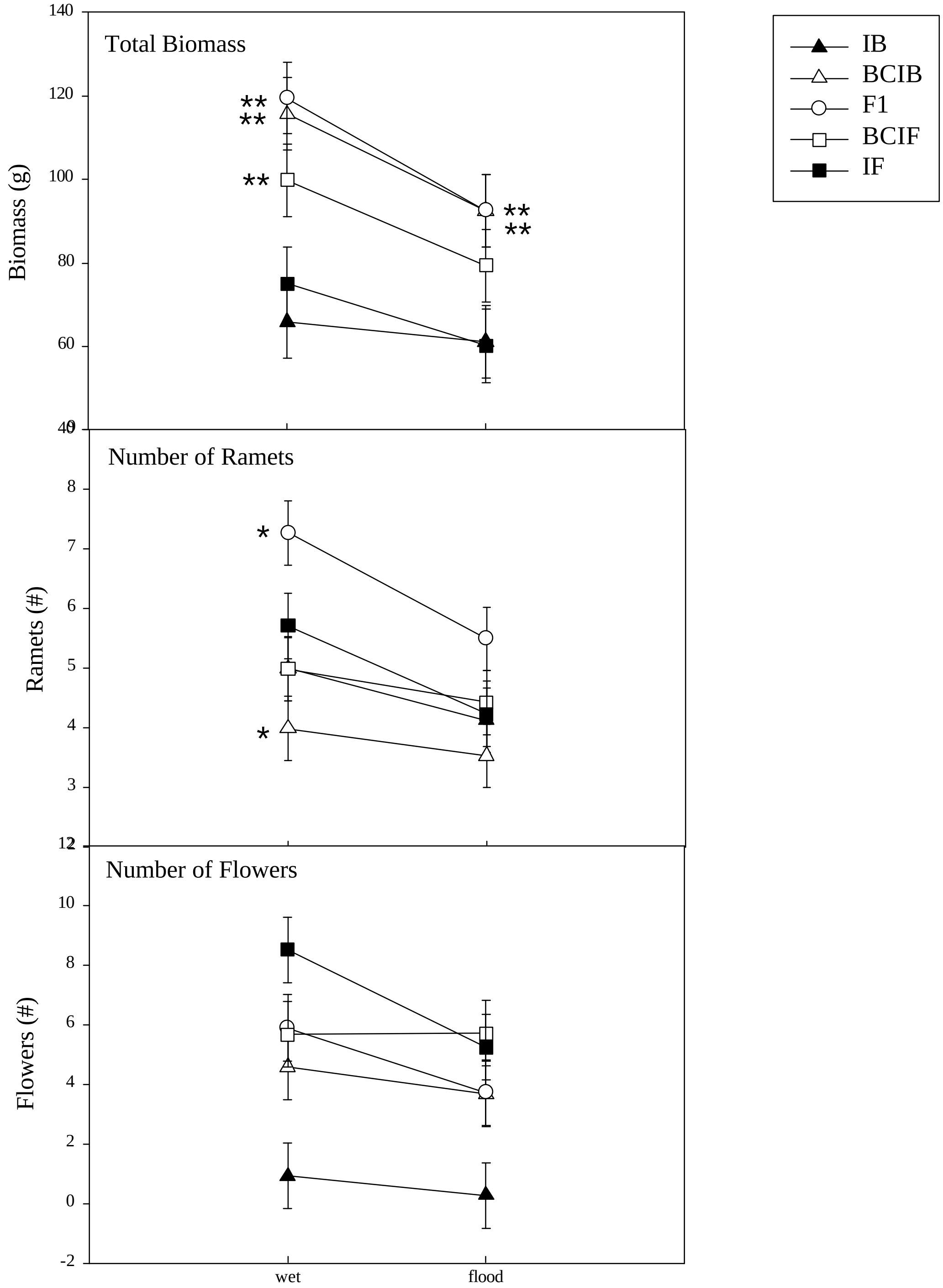

Figure 4.4. Genotypic class level fitness components (LSMEANS from SAS ± SE). Extreme hybrid classes are marked with "**" if they are significantly different from both parental species, and "*" if significantly different from both parental species (p<0.05).

exception for class-level analyses was a significant interaction for rhizome weight ratio (Table 4.1, Figure 4.2). Two classes, F_1 and BCIB allocated a greater proportion of biomass to rhizomes in the wet treatment, while BCIF, IB, and IF allocate more biomass to rhizome in the flood treatment. All classes grew more slowly and had reduced fitness in the flood treatment, but relative physiological trait expression did not change much across water treatments.

Genotype-level analyses

There were several characters for which individual genotypes displayed extreme expression. A single F_1 genotype was transgressive for top root ratio in the wet treatment (Table 4.5, Figure 4.5). Specific leaf area was transgressive in a different F_1 genotype in the wet treatment (Table 4.6, Figure 4.5). Several allocation traits were significantly different among genotypes (Table 4.6), but there was no transgressive trait expression among hybrid genotypes for these traits.

Growth and fitness characters were also transgressive in select genotypes. Growth rates varied among genotypes (Table 4.7 – genotype*time). Three hybrid genotypes (two F_1 and one BCIB) were positively transgressive for final leaf area in the wet treatment (Figure 4.6). The largest of these three was also positive transgressive in the flood treatment (Figure 4.6). The same three hybrid genotypes were transgressive for total biomass, growing bigger than all IF and IB genotypes in the wet treatment (Table 4.8, Figure 4.7). One genotype was transgressive for number of ramets (Figure 4.7).

The only trait that demonstrated a significant genotype by water interaction was number of flowers produced (Table 4.8). However, flower number did not exhibit transgressive trait expression in either watering treatment (Figure 4.7).

Table 4.5. Flood tolerance traits in Louisiana irises at the individual genotype level. There were twenty-five genotypes put into two watering treatments. Effects significant at p< 0.05 are shown in bold.

			Root allocation (g/g)		Root density (g/ml)	
Source	ndf	ddf	F	p	F	p
Genotype	24	72	2.77	**<0.001**	1.43	0.127
Water	1	14	4.34	0.056	4.25	0.058
Geno*water	24	72	1.46	0.111	1.04	0.434

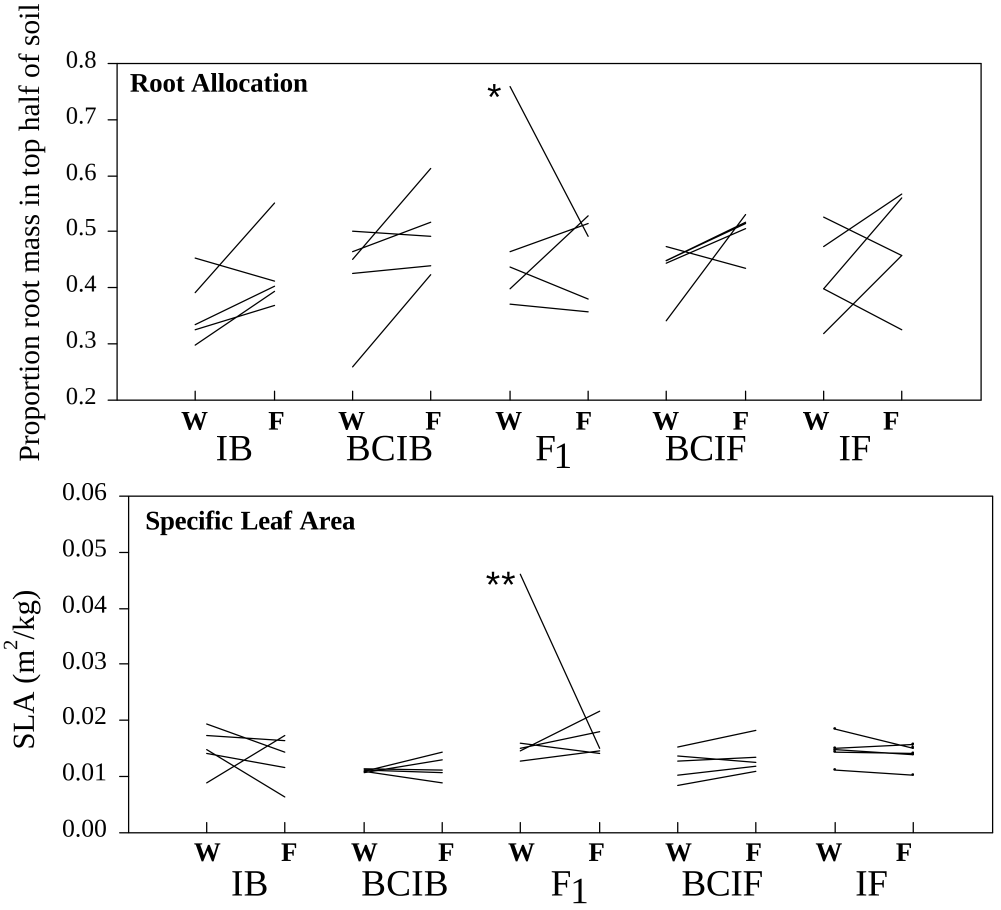

Figure 4.5. Individual genotype averages of root allocation pattern and specific leaf area in two water treatments. “W” and “F” indicate wet and flood treatment, respectively. IB and IF are *Iris brevicaulis* and *I. fulva*, and BCIB, BCIF, and F_1 are three types of hybrid cross. Asterisks indicate transgressive genotypes that are extreme and significantly different from all 10 parental species genotypes: “**”, or 8 or more parental genotypes: “*”.

Table 4.6. Biomass allocation in Louisiana irises at the individual genotype level. All data are ratios or proportions and effects significant at $p< 0.05$ are show in bold.

			Leaf area ratio (m^2/g)		Specific leaf area (m^2/g)		Leaf mass proportion (g/g)		Rhizome mass prop. (g/g)		Root mass proportion (g/g)	
Source	ndf	ddf	F	p	F	p	F	p	F	p	F	p
Genotype	24	72	2.78	**<0.001**	2.25	**0.005**	3.61	**<0.001**	1.23	0.247	5.6	**<0.001**
Water	1	14	0	0.961	0.74	0.404	0.14	0.711	1.08	0.315	30.3	**<0.001**
Geno*water	24	72	1.11	0.354	1.64	0.057	1.13	0.339	0.9	0.603	0.71	0.821

Table 4.7. Growth rate of Louisiana irises at the individual genotype level. There were 25 genotypes whose leaf area was measured 6 times over the course of the experiment. Covariate was initial rhizome fresh weight. Effects significant at $p < 0.05$ are in bold.

			Leaf area (cm^2)	
Source	ndf	ddf	F	p
covariate	1	571	41.4	**<0.001**
genotype	24	571	5.67	**<0.001**
water	1	14	6.21	**0.026**
genotype*water	24	571	1.14	0.291
time	5	571	642	**<0.001**
genotype*time	120	571	2.5	**<0.001**
water*time	5	571	3.48	**0.004**
geno*water*time	120	571	1.01	0.458

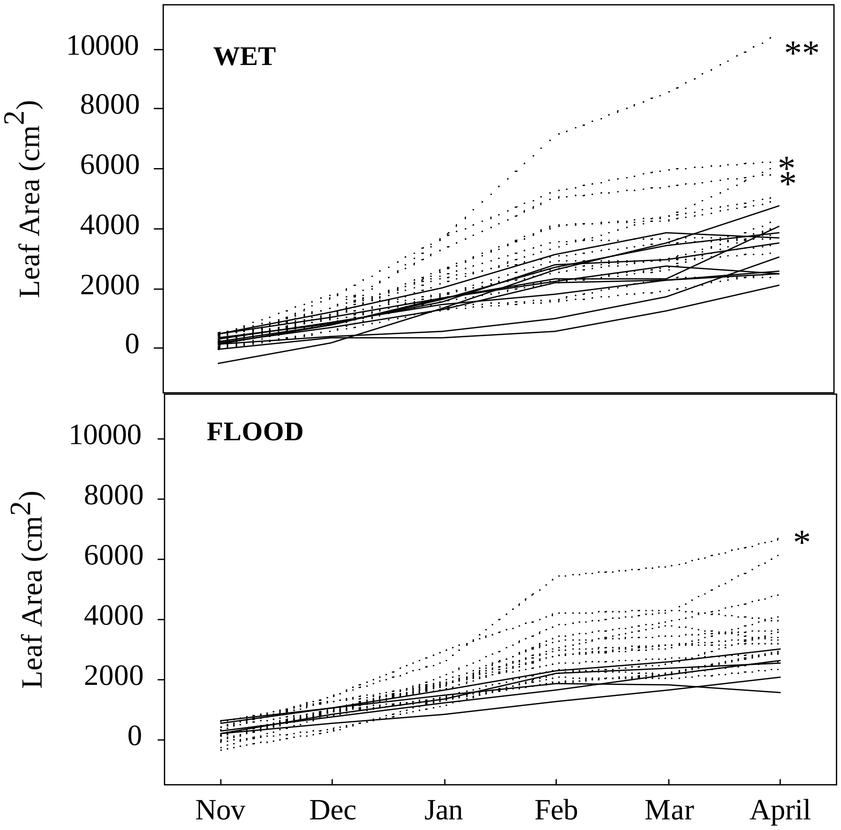

Figure 4.6. Individual genotype level growth rate in wet and flood watering treatments over the course of the experiment. The two species (IB and IF) are shown in solid lines, and all hybrid genotypes are shown as dashed lines. Asterisks indicate hybrid genotypes that are transgressive at the final measurement date. "**" indicates an extreme genotype that is significantly different from all 10 parental species, and "*" indicates significant difference from 8 or more parental genotypes.

Table 4.8. Fitness components of Louisiana irises at the individual genotype level. Effects that are significant at $p < 0.05$ are in bold.

			Total Biomass (g)		Ramets (#)		Flowers (#)	
Source	ndf	ddf	F	p	F	p	F	p
Covariate	1	71	30.4	**<0.001**	33.5	**<0.001**	7.64	**0.007**
Genotype	24	71	4.39	**<0.001**	2.67	**<0.001**	5.48	**<0.001**
Water	1	14	11.6	**0.004**	9.36	**0.009**	3.28	0.092
Geno*water	24	71	0.95	0.544	0.83	0.689	2.11	**0.008**

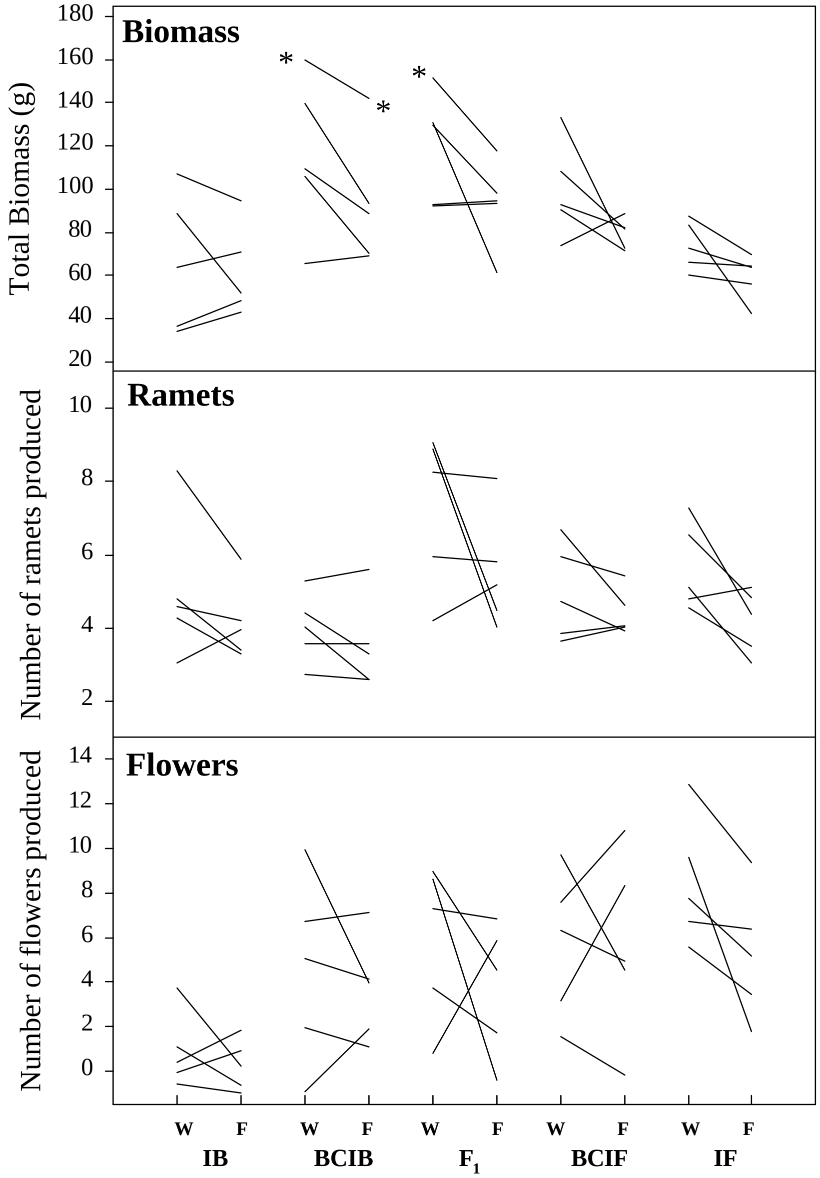

Figure 4.7. Individual genotype level fitness components for *Iris brevicaulis* and *I. fulva* (IB and IF), and three types of hybrid cross (BCIB, F_1, BCIF) in two watering treatments wet (W) and flooded (F). Total biomass and ramets are indicators of clonal fitness, and flower production is an indicator of reproductive fitness. An "*" indicates transgressive genotypes.

DISCUSSION

Our data support the paradigm that predicts hybrids can possess novel phenotypic variation in very early hybrid generations. We measured both positive and negative extreme hybrid expression of physiological and fitness related characters in two different environments at both the genotypic class and individual genotype levels. These results imply that the process of hybridization itself can generate phenotypic variation that may increase the fitness of select hybrid individuals. While genotypic class averages can reveal a tendency for an entire group of hybrids to differ relative to parental species, upon closer inspection it is often one or a few extreme genotypes that are driving the pattern. To truly understand the evolutionary potential of a hybrid swarm, physiological performance and fitness must be understood at the individual genotype-level. Our data underscore the idea that hybrid novelty will be a rare event, highly dependent on environmental context.

The transgressive expression of root allocation and specific leaf area in early generation hybrids represent potentially beneficial, ecologically relevant physiological traits that appear to be the result of recombination during interspecific hybridization. Root allocation is a trait that could affect the distribution of plants in a wetland (Coops et al. 1996; Moog 1998). By moving roots to upper more oxygenated layers of soil, plants can minimize the damage to root tissue that might result from oxygen deprivation (Armstrong et al. 1994; Ernst 1990; Justin and Armstrong 1987). The fact that a single F_1 genotype is able to allocate twice as many of its roots to the top few inches of soil in waterlogged condition may allow it to survive better than individuals of either species in an environment that is shallowly flooded for extended periods of time. Traits related to shade tolerance would affect the amount of carbon that can be fixed during a growing season, translating into rhizome mass or flower stalks. Specific leaf area, which was positively transgressive in one F_1 genotype in the wet treatment, may not relate to flood tolerance at all. However, such an increase in specific leaf area would undoubtedly have an impact on the shade tolerance of this genotype (Lambers et al. 1998; Poorter and Lambers 1986). Since the trait was only expressed in one water treatment, it would suggest that this genotype might be best suited to waterlogged, shaded conditions. The environment-dependent expression of a non-flood related trait in a flooding experiment was a bit of a surprise. However, it underscored that transgressive expression of ecologically relevant traits may require a very particular (and sometimes unexpected) environment.

Ultimately, for novel hybrid traits to affect evolutionary patterns, they must positively affect fitness. There were several cases of transgressive expression of fitness related traits in our data at both the genotypic class and individual genotype levels. Most striking was that all three hybrid classes were transgressive for total biomass, suggesting that they are all clonally more fit than *I. brevicaulis* and *I. fulva*. It seems likely that clonal habit contributes to the success of these hybrids in the wild, allowing for long-term vegetative survival, even if successful sexual reproduction is a rare event (Ellstrand et al. 1996). Fitness components measured at the genotypic class level revealed interesting differences in life history strategy between *I. brevicaulis* and *I. fulva.* While biomass and ramet number, both clonal fitness components (Wikberg 1995), were relatively similar between species, flower production was very different (Figure 4.5). One classical prediction of clonal plant biology is that flowering will be size-dependent (Harper 1977;

Mendez and Obeso 1993; Wijesinghe and Whigham 1997). Our dataset, taken as a whole, do not support this expectation. Regardless of size, *I. brevicaulis* plants flowered very little and *I. fulva* produced many on average, suggesting that *I. fulva* has a more sexual reproductive strategy, while *I. brevicaulis* relies more on clonal reproduction. The most valuable hybrid novelty in terms of reproductive strategy may be in the combination of traits inherited from the two parents. Some hybrid genotypes appear to be strong clonally and sexually, agreeing with earlier studies of pollen and seed viability (Burke et al. 1998b). In the wild, where resources are more limiting there may be a cost to excessive clonal and sexual reproduction. However, under controlled conditions, many hybrid genotypes and genotypic classes appear to be very fit relative to parents.

The actual fitness value of any individual trait can vary significantly by environment (Dudley 1996). Traits such as root allocation to surface layers of soil may be advantageous while the soil in inundated, but be a liability when the water table drops (Ernst 1990). Nearly all individuals with transgressive traits expressed them only in one of the two water treatments. Phenotypes at the individual genotype level reveal a large amount of genotype by environment response (for example Figure 4.7). Some of the most fit individual genotypes in one environment were among the least fit in the other, suggesting that the right ecological opportunity is critical for the expression and success of novel hybrid traits. Thus, our data do support environment-dependence as a factor in hybrid phenotypic trait expression and fitness. Wetlands, like many environments, contain a high degree of microhabitat heterogeneity (Huenneke and Sharitz 1986; Snow and Vince 1984) and are therefore rich in possibilities for genotype by environment interactions.

Both genotypic class and individual genotype analyses suggest that among the genotypes present in this study, there were detectable extreme hybrid phenotypes for traits of potential ecological importance. Genotypic class averages were useful as indicators of general trends in hybrid trait expression and fitness. However, averaging masked some extreme phenotypes, and caused some genotypic class averages to appear extreme, when only one extreme genotype was present. If rare recombinational events give rise to traits that might permit survival in extreme environments, leading to rapid ecological divergence (Rieseberg 1997), it is the rare, extreme phenotype that is most interesting. Observing genotypes that were positive transgressive for final biomass was convincing evidence that early generation hybrids between Louisiana irises may enjoy environment-dependent increases in fitness over both parental species. Early generation hybrids between *I. brevicaulis* and *I. fulva* have been shown to have pollen viability and fertility that rivals the parent species (Burke et al. 1998a). In the present study it is plain that some hybrids excel at growth and production of clonal ramets. Additionally, as rhizomatous perennials, these hybrids appear to be able to survive many years and expand across several microhabitat types (Burke et al. 2000b). It is possible that as they spread spatially, individual genotypes may be capable of "specializing" on habitat patches in which they flourish (Bazzaz 1991). If hybrids that possess ecologically relevant transgressive traits are able to colonize several types of habitat during their lifetime, the odds of encountering habitat to which they might be uniquely suited will increase.

ACKNOWLEDGEMENTS

The authors wish to thank, A. Tull, M. Boyd, and M. Zimmerman for keeping the plants healthy and happy during the course of this experiment. We wish to especially acknowledge C. Patterson who spent many hours collecting data in the greenhouse. Thanks to the harvesting crew, including M. Phillips, R. Parakesh, A. Bouck, V. Jin, S. Crockett, C. Richards, S. Franks, J. West, J. Espeleta, E. Kentner and R. Addington. We want to thank A. Bouck, D. Rosenthal, K. Snyder, J. West, S. Cornman, and E. Kentner for comments on earlier versions of the manuscript. This research was supported by NSF grant # DEB-0074159 to MLA.

LITERATURE CITED

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378-388.

Armstrong, W., R. Brandle, and M. B. Jackson. 1994. Mechanisms of Flood Tolerance in Plants. Acta Botanica Neerlandica 43:307-358.

Arnold, M. L. 1992. Natural Hybridization as an Evolutionary Process. Annual Review of Ecology and Systematics 23:237-261.

Arnold, M. L. 1994. Natural Hybridization and Louisiana Irises. Bioscience 44:141-147.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Arnold, M. L. 2000. Anderson's paradigm: Louisiana Irises and the study of evolutionary phenomena. Molecular Ecology 9:1687-1698.

Arnold, M. L., E. K. Kentner, J. A. Johnston, S. Cornman, and A. C. Bouck. 2001. Natural hybridisation and fitness. Taxon 50:93-104.

Barton, N. H. 2001. The role of hybridization in evolution. Molecular Ecology 10:551-568.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16:113-148.

Bazzaz, F. A. 1991. Habitat Selection in Plants. American Naturalist 137:S116-S130.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the Fitness of Hybrids. Annual Review of Genetics 35:31-52.

Burke, J. M., M. R. Bulger, R. A. Wesselingh, and M. L. Arnold. 2000a. Frequency and spatial patterning of clonal reproduction in Louisiana iris hybrid populations. Evolution 54:137-144.

Burke, J. M., M. R. Bulger, R. A. Wesselingh, and M. L. Arnold. 2000b. Frequency and spatial patterning of clonal reproduction in Louisianan *Iris* hybrid populations. Evolution 54:137-144.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998a. Hybrid fitness in the Louisiana irises: Analysis of parental and F1 performance. Evolution 52:37-43.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998b. Hybrid fitness in the Louisiana irises: Analysis of parental and F-1 performance. Evolution 52:37-43.

Campbell, D. R., and N. M. Waser. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669-676.

Coops, H., F. W. B. vandenBrink, and G. vanderVelde. 1996. Growth and morphological responses of four helophyte species in an experimental water-depth gradient. Aquatic Botany 54:11-24.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and Genetic Associations in an Iris Hybrid Zone. Evolution 47:1432-1445.

Curran, M., P. James, and W. G. Allaway. 1996. The measurement of gas spaces in the roots of aquatic plants - Archimedes revisited. Aquatic Botany 54:255-261.

de Vicente, M. C., and S. D. Tanksley. 1993. Qtl Analysis of Transgressive Segregation in an Interspecific Tomato Cross. Genetics 134:585-596.

Devicente, M. C., and S. D. Tanksley. 1993. Qtl Analysis of Transgressive Segregation in an Interspecific Tomato Cross. Genetics 134:585-596.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. The American Naturalist 74:312-321.

Dudley, S. A. 1996. Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypotheses. Evolution 50:92-102.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America 97:7043-7050.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Sciences of the United States of America 93:5090-5093.

Ernst, W. H. O. 1990. Ecophysiology of Plants in Waterlogged and Flooded Environments. Aquatic Botany 38:73-90.

Gardner, S. N., and M. Mangel. 1999. Modeling investments in seeds, clonal offspring, and translocation in a clonal plant. Ecology 80:1202-1220.

Grant, B. R., and P. R. Grant. 1996. High survival of Darwin's finch hybrids: Effects of beak morphology and diets. Ecology 77:500-509.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, San Diego, CA, USA.

Harrison, R. G. 1986. Pattern and Process in a Narrow Hybrid Zone. Heredity 56:337-349.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution 53:866-873.

Hewitt, G. M. 1988. Hybrid Zones - Natural Laboratories for Evolutionary Studies. Trends in Ecology & Evolution 3:158-167.

Howard, D. J. 1986. A Zone of Overlap and Hybridization between 2 Ground Cricket Species. Evolution 40:34-43.

Huenneke, L. F., and R. R. Sharitz. 1986. Microsite Abundance and Distrbiution of Woody Seedlings in a South-Carolina Cypress-Tupelo Swamp. American Midland Naturalist 115:328-335.

Johnston, J. A., D. J. Grise, L. A. Donovan, and M. L. Arnold. 2001a. Environment-dependent performance and fitness of Iris brevicaulis, I-fulva (Iridaceae), and hybrids. American Journal of Botany 88:933-938.

Johnston, J. A., R. A. Wesselingh, A. C. Bouck, L. A. Donovan, and M. L. Arnold. 2001b. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana Iris hybrid population. Molecular Ecology 10:673-681.

Justin, S., and W. Armstrong. 1987. The Anatomical Characteristics of Roots and Plant-Response to Soil Flooding. New Phytologist 106:465-495.

Lambers, H., F. S. Chapin, and T. L. Pons. 1998. Plant Physiological Ecology. Springer-Verlag, New York, NY, USA.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315-&.

Littell, R. C., G. A. Milliiken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute, Cary, NC, USA.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Mendez, M., and J. R. Obeso. 1993. Size-Dependent Reproductive and Vegetative Allocation in Arum- Italicum (Araceae). Canadian Journal of Botany-Revue Canadienne De Botanique 71:309-314.

Moog, P. R. 1998. Flooding tolerance of Carex species. I. Root structure. Planta 207:189-198.

Moore, W. S. 1977. Evaluation of Narrow Hybrid Zones in Vertebrates. Quarterly Review of Biology 52:263-277.

Poorter, H., and H. Lambers. 1986. Growth and Competitive Ability of a Highly Plastic and a Marginally Plastic Genotype of Plantago-Major in a Fluctuating Environment. Physiologia Plantarum 67:217-222.

Rieseberg, L. H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg, L. H., and N. C. Ellstrand. 1993. What Can Molecular and Morphological Markers Tell Us About Plant Hybridization. Critical Reviews in Plant Sciences 12:213-241.

Riley, H. P. 1938. A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. American Journal of Botany 25:649-725.

SAS. 1989. SAS/STAT user's guide, version 6. SAS Institute, Cary, NC.

Schwarzbach, A. E., L. A. Donovan, and L. H. Rieseberg. 2001. Transgressive character expression in a hybrid sunflower species. American Journal of Botany 88:270-277.

Snow, A. A., and S. W. Vince. 1984. Plant Zonation in an Alaskan Salt-Marsh .2. An Experimental- Study of the Role of Edaphic Conditions. Journal of Ecology 72:669-684.

Viosca, P. 1935. The irises of Southeastern Louisiana: a taxanomic and ecological interpretation. Bulletin of the American Iris Society 57:3-56.

Wesselingh, R. A., and M. L. Arnold. 2000. Pollinator behaviour and the evolution of Louisiana iris hybrid zones. Journal of Evolutionary Biology 13:171-180.

Wijesinghe, D. K., and D. F. Whigham. 1997. Costs of producing clonal offspring and the effects of plant size on population dynamics of the woodland herb Uvularia perfoliata (Liliaceae). Journal of Ecology 85:907-919.

Wikberg, S. 1995. Fitness in Clonal Plants. Oikos 72:293-297.

Wikberg, S., B. M. Svensson, and B. A. Carlsson. 1994. Fitness, Population-Growth Rate and Flowering in Carex- Bigelowii, a Clonal Sedge. Oikos 70:57-64.

CHAPTER 5

HIGH HYBRID FITNESS AT SEED AND SEEDLING LIFE HISTORY STAGES IN LOUISIANA IRISES[1]

---

[1] Johnston, J. A., M. L. Arnold, and L. A. Donovan. To be submitted to *Evolution*.

ABSTRACT

Relative fitness of hybrid genotypes will determine how large a role that hybrids can play in the evolution a plant species complex. To realistically evaluate hybrid fitness in a long-lived species, fitness at all likely selection bottlenecks must be measured. Many long-lived plants have high mortality at the seed and seedling stages, making this a likely stage for hybrid genotypes to be disproportionately removed from a population. We evaluated germination, seedling survival and growth of two Louisiana Iris species and their early generation hybrids in several experimental environments, to test for a hybrid fitness bottleneck at these early stages. Manipulation of light and water conditions revealed that species and hybrids require similar conditions for seed germination: wet but well drained. Germination was more frequent in shaded treatments, but seedling survival was higher in full sun. *Iris brevicaulis* exhibited the lowest germination and seedling survival overall, yet grew vigorously. *Iris fulva* had high levels of germination and seedling survival, yet yielded the smallest plants at the end of one season of growth. Hybrid classes had rates of germination, seedling survival, and seedling growth that equaled or exceeded the strongest parent, indicating that hybrids are generally as fit, if not more fit, than parents at seed and seedling stages, regardless of environmental conditions. Our findings suggest that *Iris fulva* and *I. brevicaulis* share a regeneration niche with each other and their hybrids, and that there is not a hybrid fitness bottleneck at seed or seedling stages.

INTRODUCTION

Individuals from many plant species can tolerate a large range of environmental conditions as adults, while requiring a specific narrow range of conditions for successful seed germination and seedling establishment (Grubb 1977; Hobbs and Mooney 1985). Closely related species can have very different requirements for germination (Harper et al. 1965), which can ecologically distinguish species that are ecologically similar as adults (Grubb 1977). When different ecological selection factors act on seed germination and seedling survival, selection at the latter stage will alter or refine patterns begun by spatially patchy germination (Martens et al. 2001; Noe and Zedler 2001). Thus within the realm of the regeneration niche, seeds and seedlings can have conflicting ecological requirements, resulting in strong selection at both stages (Schupp 1995). If germination requirements are specific (Harper et al. 1965), and seedling establishment is restricted to a narrow (and potentially different) range of abiotic factors (Grubb 1977; Kuuluvainen and Juntunen 1998), genetic changes that affect characteristics of the "regeneration niche" could drive ecological divergence of populations.

Hybridization is gaining stature as a creative evolutionary force in wild plant populations (Anderson and Stebbins 1954; Arnold 1997; Rieseberg 1997) following several decades of skepticism by evolutionary biologists (Dobzhansky 1940; Mayr 1942; Wagner 1970). While hybrids have long been viewed by plant breeders as a source of phenotypic innovations, only recently has the evolutionary value of hybrid novelty been explored in natural populations (Bennett and Grace 1990; Grant and Grant 1992; Orians 2000). Some hybrid genotypes will inevitably have low fitness due to genetic complications, but hybrid fitness is usually highly variable (Cruzan and Arnold 1994;

Rosenthal et al. In Press) including some relatively fit genotypes that have the potential to contribute new genetic variation to future generations (Burke and Arnold 2001; Emms and Arnold 1997; Grant and Grant 1996). Environmental context and life history traits are often important components of hybrid success (Arnold and Hodges 1995; Rieseberg et al. 1999). Many documented cases of hybridization involve plant taxa that are long-lived and can reproduce via sexual and vegetative means (Ellstrand et al. 1996). Without the need for frequent success with sexual reproduction, taxa that can reproduce clonally can avoid the potentially strong selection at seed and seedling stage, facilitating the persistence of hybrid genotypes in a population (Grant 1981).

Adult viability and reproductive fitness components have been extensively studied in Louisiana irises, a group of naturally hybridizing clonal plants whose distributions overlap in southern Louisiana. Experimental hybrids between two species, *Iris fulva* Ker-Gawler and *Iris brevicaulis* Walter (IRIDACEAE) are the focus of the current study. Reproductively, hybrids produce flowers that combine the long lifespan of *I. fulva* and the high nectar concentration of *I. brevicaulis* (Wesselingh and Arnold 2000), resulting in attractive targets for pollinators. Hybrid pollen is successful at fertilizing parent species and generating viable backcrossed offspring (Cruzan and Arnold 1994). Hybrid fitness is generally very high for established adult Louisiana irises, but relative hybrid performance changes in different environmental conditions such as drought or deep shade (Johnston, Donovan, and Arnold, *unpublished data*). While F_1's are rare in natural populations due to a cascade of reproductive barriers between species, once a hybrid genotype becomes established in a population, it can act as a bridge that facilitates further gene movement between species (Arnold 2000). As hybrids backcross toward parents, beneficial traits that arose during hybridization can be integrated into the genetic background of one parent species, potentially increasing fitness for the entire population (Anderson and Stebbins 1954; Arnold 1992; Ellstrand and Schierenbeck 2000).

If environment-dependent hybrid fitness is the key to understanding the ecological impacts of hybridization in natural plant populations, selection at early life history stages cannot be ignored. By exploring seed germination, seedling survival and growth of hybrids between *I. brevicaulis* and *I. fulva* in several experimental environments, a major gap in our understanding of relative hybrid fitness at these early life history stages can be filled. In this study we will address two specific questions. First, are hybrids as fit as parental species in terms of seed germination and seedling growth and survival? Second, does relative hybrid fitness at seed and seedling stages change across environmental conditions? Finally, we compare fitness of hybrids and parents at early life stages to adult fitness obtained in previous studies, and examine relative hybrid *Iris* fitness from seed to adult.

METHODS

Study species biology

Iris brevicaulis and *I. fulva* are obligate wetland plants, occurring along the edges of bayous and swamps in throughout their ranges (Viosca 1935). Water and light are the primary and secondary factors that distinguish habitat of the two species (Johnston et al. 2001b). *Iris brevicaulis* lives just above mean water line in slightly drier, sunnier habitat patches than *I. fulva* (Cruzan and Arnold 1993; Johnston et al. 2001b). In southern Louisiana, these Iris species occur in sparsely shade patches that are often waterlogged but rarely flooded for long periods of time (Cruzan and Arnold 1993). In these areas, hybrid zones can form (Arnold 1994) but are not reported elsewhere.

Seed germination experiment

Four genotypic classes of seeds were used in a germination experiment, *Iris brevicaulis* (IB) and *Iris fulva* (IF), and two types of hybrid cross. Hybrid crosses were initiated in 1996, when IB and IF were artificially cross-pollinated to create F_1 hybrid progeny. Both IF and IB served as maternal parents of hybrid seeds. F_1 hybrids were selfed in 1997 to produce the second filial, or F_2 generation hybrids used in this experiment. Hybrids that initially had IB maternal parents will be referred to as F_2B, and hybrids from IF maternal parents are F_2F. All seeds used in this experiment were produced during the 1997 growing season.

The germination experiment was carried out in the Botany greenhouses at the University of Georgia, Athens, GA, USA. Light and water conditions were experimentally manipulated to measure the effects of each environmental treatment combination on seed germination of all four genotypic classes described above. Seeds were planted in pots with nine 5 cm^3 compartments, which allowed the manipulation of environmental conditions on eight seeds at a time (leaving one compartment empty). Flats were filled with potting soil, and seeds were buried until just below the soil surface. The experimental design was a split-split plot. The split plot factor was light, and the split-split plot factor was water. There were three levels of light: sun (100% ambient), shade (50% ambient), and deep shade (25% ambient), similar to those used by Bennett and Grace 1990). Light treatment levels were achieved by stretching neutral shade cloth on a PVC frame that was suspended a few centimeters above the soil surface. Three water treatments were used: field capacity (watered but well drained), flooded (submerged to soil surface), and fluctuating (alternating between flooded and field capacity, every four weeks). Each 9-hole flat insert was placed in a plastic tub designed to impose one of the three water treatments.

With four genotypic classes (IB, F_2B, F_2F, IF), three light treatments (sun, shade, deep shade), three water treatments (field capacity, flooded, fluctuating), there were 36 treatment combinations in this experiment. Each combination is replicated two times per experimental unit or block, and the block is replicated 10 times on a greenhouse bench (see Figure 5.1a), for a total of 720 seeds. Germination was monitored and recorded twice weekly from May 15, 1999 to December 15, 1999. As they germinated, plants were removed from the experimental array to prevent density-dependent effects. At the end of the experiment, all ungerminated seeds were broken open and examined to evaluate their viability. Solid, white seeds were considered viable, while black, slimy, or hollow seeds were considered inviable. Germination proportions for seeds in each treatment combination were calculated for each block. Proportions were arcsine square root transformed and analyzed in SAS PROC MIXED, to explicitly model the random effect block.

Seedling growth experiment

An experiment to measure growth and survival of seedlings was conducted using additional seeds from the crosses described above. On October 31, 2000, seed scarification began to synchronize germination. Seeds were soaked in water for 24 hours, then in 4% hydrogen peroxide for 30 minutes (Kindiger 1994) before scarification. Seeds were removed from their corky, maternally derived outer layer. The seed coat was cut

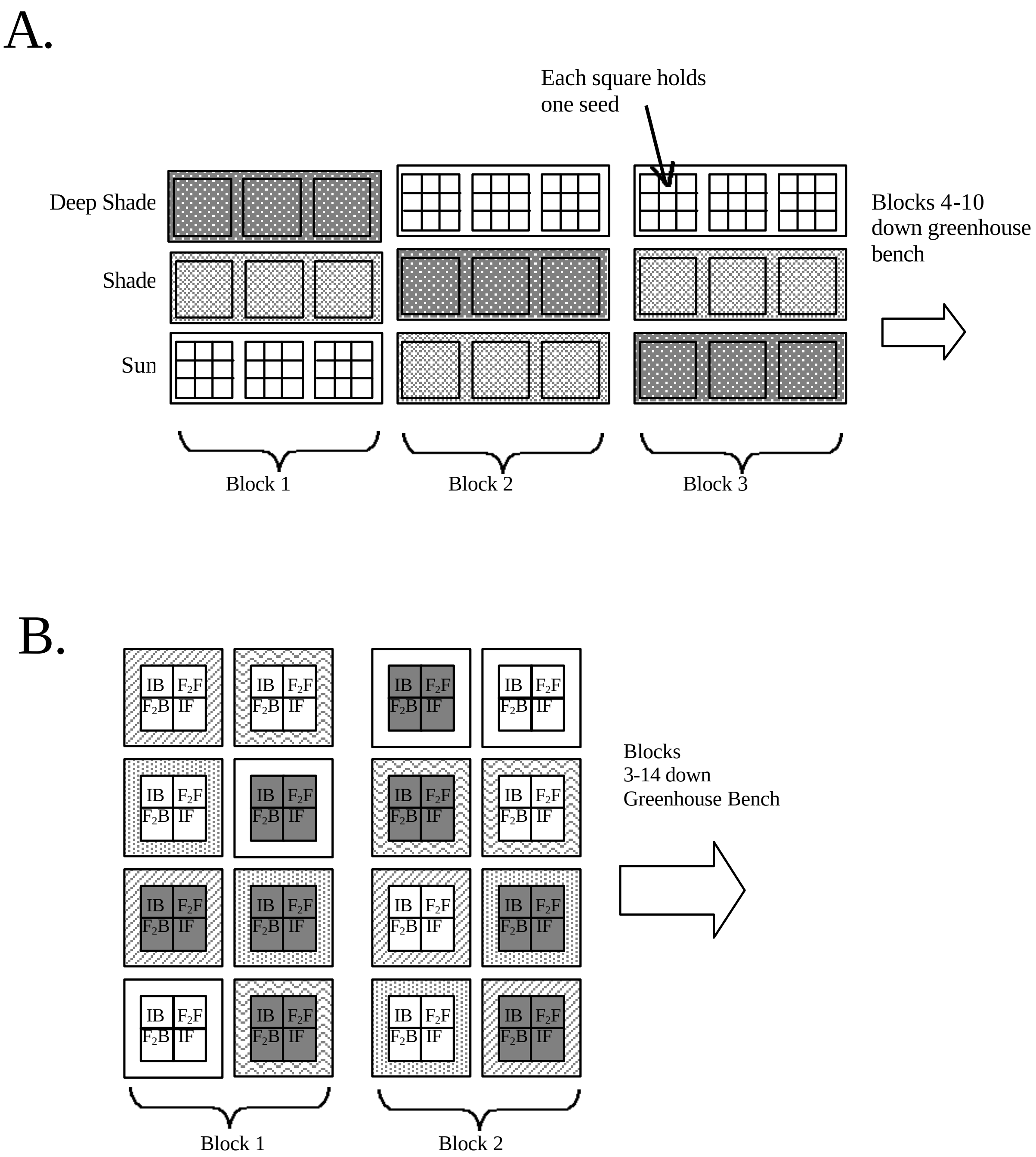

Figure 5.1. Experimental setup of seedling germination (a) and seed growth and survival experiments (b). For the germination experiment, light treatment split plots (deep shade, shade, sun) are represented by filled or open rectangles, and each large square represents a 9-hole flat insert that is placed in its own water treatment tray (field capacity, flood, fluctuating). In the seedling growth and survival experiment, four pots containing one seedling of each type were independently assigned a water treatment (dry, optimal, flooding, and fluctuating - represented by fill pattern), and sun or shade treatment (open or dark inner square). In both cases, the experimental unit was replicated on a greenhouse bench (10 reps germination, 14 reps growth).

with a razor blade, and a small piece of the endosperm was removed to expose the embryo (Stoltz 1968). Twenty scarified seeds were placed on 130 mm Petri plates containing ~50 ml agar mixed with fertilizer. (For every 1 L water, 2.54 g Peters' fertilizer (20-20-10) and 10 g bacto-agar were mixed and autoclaved for 20 minutes, then poured.) Plates were sealed and left on a lab bench receiving indirect, natural light for one week. When both root and shoot were visible, seeds were removed from plates and placed in individual cups containing 10 ml of fresh growth medium. Cups were sealed in plastic deli containers to prevent desiccation. Three weeks after scarification, all available seedlings were transplanted into 5 cm square pots filled with a mixture of equal parts sand and native soil brought from St. Martin Parish, Louisiana, USA. From the seedlings that survived transplant shock, individuals were randomly chosen and assigned to experimental treatments. Experimental treatment began on December 15, 2000.

Light and water were manipulated in the seedling growth experiment. Four levels of water were used: dry, optimal, flooded, and fluctuating. All plants were watered with a drip tube system that provided one minute of irrigation once a day. Differences in water treatment were achieved by manipulating the plastic container in which seedlings sat. Dry treatment containers had pinholes in the bottom, optimal containers had holes 1 cm from the bottom, flooded containers had no holes, and retained water to the surface of the soil. The fluctuating treatment alternated between dry and flooded by changing the plastic container on a 4-week rotation. Two levels of light were used, sun and shade. Sun treatment plants were given full ambient light, and shade plants were placed under small cloth tents (see Figure 5.1b). The light level in the shade treatment was about 10% ambient.

The experiment was arranged in a split-plot design, with light and water treatment combination as the split-plot factor, and genotypic class as the sub-plot factor. Light and water treatment combinations were assigned at random to sets of four seedlings, one each of IB, F_2B, F_2F, and IF. With four levels of genotypic class, four levels of water and two levels of light, there were 32 treatment combinations in all. Within an experimental unit, each combination was present once, and there were 14 replications of the experimental unit across a greenhouse bench.

During the course of the experiment, leaf area was measured three times at seven-week intervals (12/15 – treatment initiation, 02/02, and 03/27-termination of the experiment) as a non-destructive estimate of growth rate. The length of every visible leaf was measured to the nearest cm, and width at (midpoint) to the nearest mm. Leaf area was estimated by calculating the area of the resulting rectangle. A previous study (Johnston et al. 2001a) indicated that this is a reasonable estimate of leaf area for adult irises. At final harvest, leaves of all plants were run through a LI-3000 leaf area meter (Li-Cor, Lincoln, NE, USA) to verify our leaf area estimates. Correlation between actual and estimated leaf areas was high ($r^2 = 0.83$). Following harvest, leaves, rhizome (if present), and roots were separated and dried for 48 h at 60 °C. Seventy seedlings died during the course of the experiment, prompting an evaluation of seedling mortality.

Growth and total biomass were analyzed in PROC MIXED in SAS, with block, as the random effect. Growth was analyzed as split-plot, repeated measures ANOVAs with first order autoregressive correlation structure. Raw growth data violated the assumption of normality, so square root transformed data were used in the final analysis. Total biomass was analyzed as split-plot ANOVA in PROC MIXED. Seedling survival was analyzed as proportional mortality. Because the experiment was not initially designed to measure mortality, experimental replicates were grouped to form artificial blocks. Proportional mortality (# dead / total in each "block") was analyzed using an ANOVA, after proportions were arcsine square root transformed.

RESULTS

Seed germination experiment

Seed germination was greatly influenced by environmental conditions, but demonstrated similar patterns across genotypic classes. A total of 99 seeds germinated out of 720 planted for the experiment, all of them in the field capacity (watered, but well-drained) treatment. Germination data from the field capacity treatment were analyzed for effects of genotypic class and light. Germination was strongly influenced by light environment and genotypic class (Table 5.1). More seeds germinated in the two shade treatments than in sun (deep shade *vs.* sun $p < 0.001$, shade *vs.* sun $p = 0.001$). The interaction between genotypic class and light was nearly significant (Table 5.1), so average germination is shown for each class in each water treatment (Figure 5.2). In the shade treatments more seeds germinated from IF than from IB. Both hybrid classes tended to be intermediate (Figure 5.2).

At the termination of the experiment, only three of the 621 ungerminated seeds were definitely inviable. The remainder were solid and appeared to be solid and healthy.

Seedling growth and survival

In the seedling growth and survival experiment, 70 out of 448 seedlings died due to the experimental conditions. Four seedlings died due to equipment failure, and were eliminated from the analyses. Genotypic class, water, and light all had significant main

Table 5.1. ANOVA table for proportion of seeds that germinated in each of four genotypic classes of Louisiana irises. *Iris brevicaulis*, *I. fulva*, and two types of F_2 hybrids were used tested for their germination in full sun, shade, and deep shade.

Source	ndf	ddf	F	p
class	3	81	4.21	**0.008**
light	2	18	14.3	**<0.001**
class*light	6	81	1.97	0.079

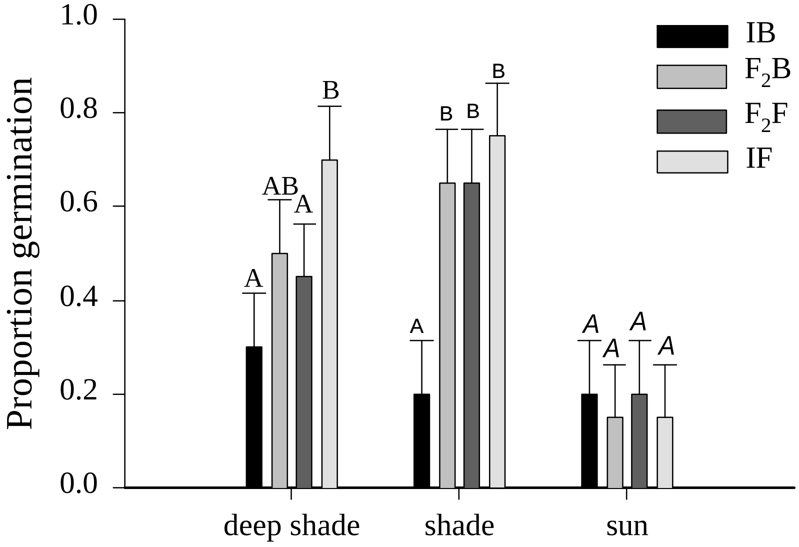

Figure 5.2. Proportion of seeds that germinated in four genotypic classes of Louisiana Iris (IB – *I. brevicaulis*, IF – *I. fulva*, and two F_2 hybrid crosses). All data shown were from the field capacity water treatment, and are separated by light. Letters indicates results from means comparisons within each light treatment.

effects on mortality (Table 5.2). High mortality in all genotypic classes in the dry, shade treatment drove a significant interaction between water and light (Figure 5.3a). While there were not any interactions between genotypic class and either environmental factor, over all treatments IB suffered significantly higher mortality than any of the other genotypic classes (Figure 5.3b).

Among the seedlings that survived, growth responded strongly to environmental factors, especially light. Sun and shade plants grew at such different rates that they were analyzed as separate data sets (see Figure 5.4). Growth rates in sun plants differed among genotypic classes and water treatments (significant class*time, water*time in Table 5.3). The F_2B hybrid class grew to have the largest leaf area (LSMEAN ± SE = 14.0 ± 0.25 cm^2), and was significantly larger than IF, the smallest class (13.3 ± 0.25 cm^2, $p = 0.047$). Among watering treatments, the dry treatment plants grew the largest, and were significantly different from optimal and flooded treatments, with the fluctuating water treatment intermediate (Figure 5.4). Among shade plants, growth rate did not differ among genotypic classes (class*time n.s., Table 5.3). Water was the only factor that significantly affected growth rate, with optimal plants growing significantly faster than all other groups (Figure 5.4).

Both genotypic class and water treatment had significant effects on total biomass of plants in the sun treatment (Table 5.4). The F_2B genotypic class had the largest average biomass, and was significantly bigger than IF (Figure 5.5), while IB and F_2B tended to be intermediate. Biomass patterns among watering treatments were similar to final leaf areas (dry > fluctuating = optimal ≥ flooded). Total biomass in shaded plants was not significantly different among genotypic classes or water treatments (Table 5.5).

Table 5.2. ANOVA table from analysis of the proportional mortality in 444 Louisiana Iris seedlings. Four genotypic classes, *I. brevicaulis*, *I. fulva*, and two F_2 hybrid crosses were subjected to combinations of experimental shade and water treatments and monitored for one growing season. Seventy seedlings died during the experiment.

Source	ndf	ddf	F	p
class	3	93	6.96	**<0.001**
water	3	93	4.36	**0.006**
light	1	93	19.93	**<0.001**
class*water	9	93	0.57	0.820
class*light	3	93	0.84	0.475
water*light	3	93	9.2	**<0.001**
class*water*light	9	93	0.78	0.640

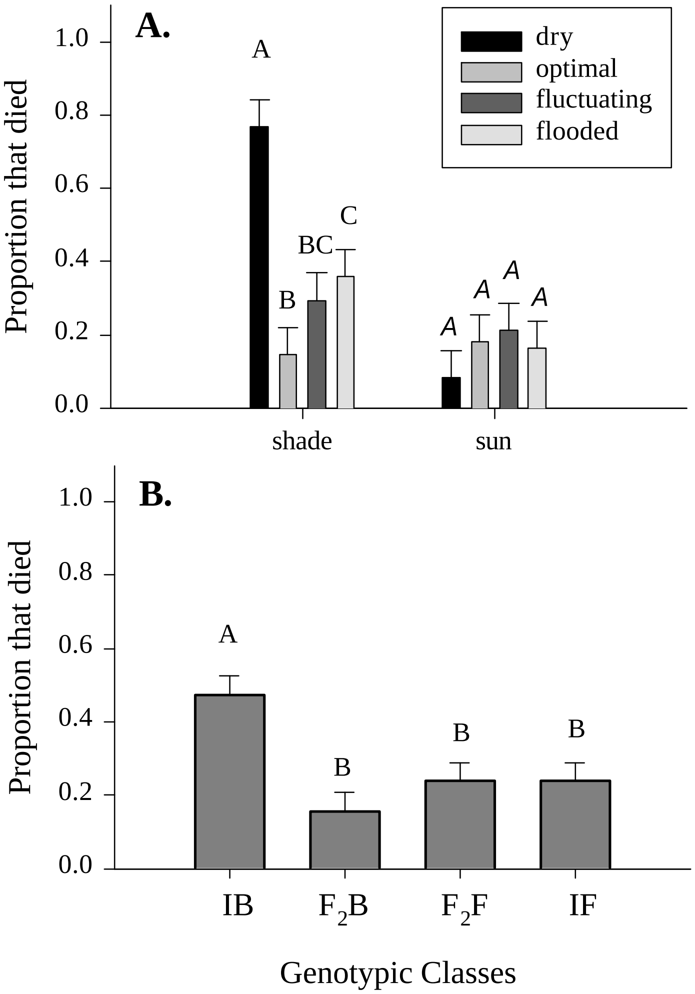

Figure 5.3. Mortality among Louisiana Iris seedlings during an experiment designed to determine growth patterns in response to light and water manipulations. Mortality data are shown as proportions, and grouped in two different ways: by environmental treatment (A) and genotypic class: IB – *I. brevicaulis*, IF – *I. fulva*, and two F_2 hybrid crosses (B). Letters above bars denote significant differences among water treatments within light treatments (A) and among genotypic classes (B).

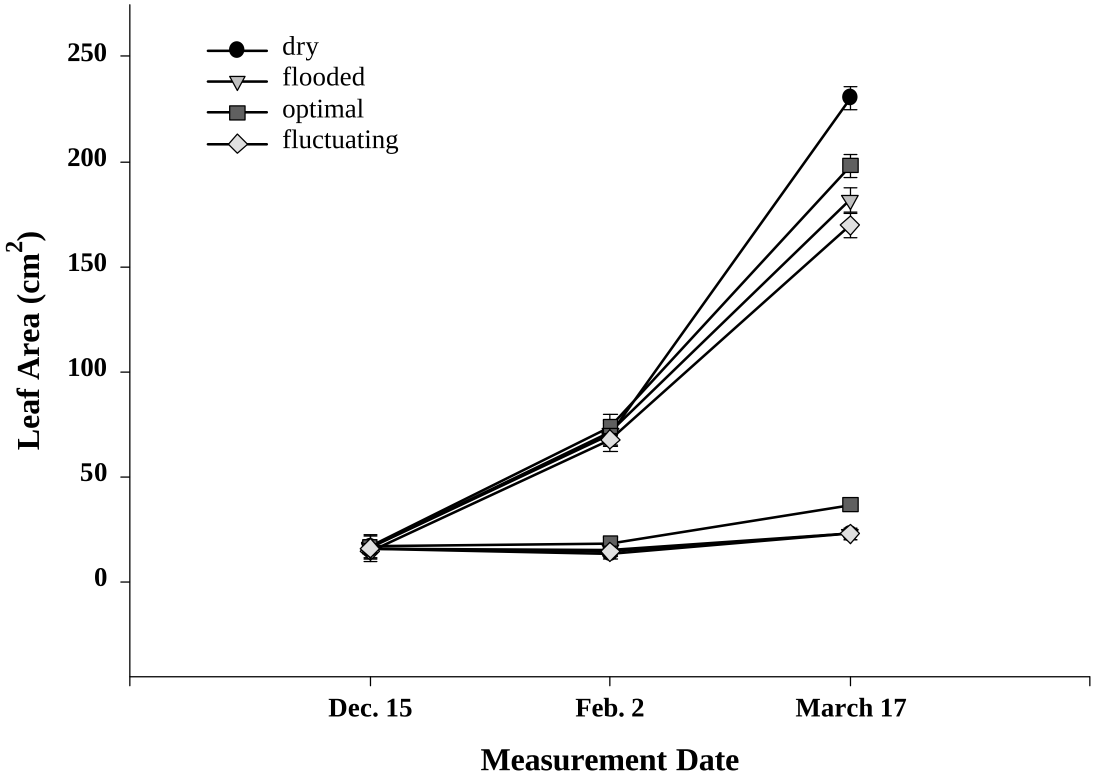

Figure 5.4. Growth rates for four genotypic classes of Louisiana Iris, *I. brevicaulis, I. fulva*, and two F_2 hybrid crosses in four water treatments, dry, flooded, and fluctuating, and two light treatments, sun and shade. Plants grown in the sun (upper curves) were analyzed separately from plants grown in the shade (lower curves). There was a significant interaction of water treatment by time in the sun plants, indicating a difference in growth rate for plants in different water treatments.

Table 5.3. Repeated measures ANOVA of leaf area in four genotypic classes of Louisiana Iris seedling. *Iris brevicaulis, I. fulva*, and two types of F_2 hybrid cross were subjected to combinations of light and water treatment, and followed for an entire growing season. Leaf area was measured at three points in the experiment, as a non-destructive estimate of plant size and analyzed as a measure of growth.

	Sun				Shade			
Source	ndf	ddf	F	p	ndf	ddf	F	p
class	3	536	1.6	0.189	3	475	1.95	0.121
water	3	39	3.55	**0.023**	3	38	1.02	0.394
time	2	536	3362	**<0.001**	2	475	92.2	**<0.001**
class*water	9	536	0.97	0.464	9	475	1.07	0.381
class*time	6	536	2.8	**0.011**	6	475	1.9	0.079
water*time	6	536	11.4	**<0.001**	6	475	2.15	**0.047**
class*water*time	18	536	0.93	0.543	18	475	0.64	0.865

Table 5.4. ANOVA tables from analysis of total biomass of Louisiana Iris seedlings at the end of one growing season. Both water and light were experimentally manipulated, and the two light treatments were analyzed separately due to extreme differences in seedling size in sun and shade.

Total Biomass	Sun				Shade			
Source	ndf	ndf	F	p	ndf	ddf	F	p
class	3	139	3.02	**0.032**	3	103	0.25	0.850
water	3	39	4.91	**0.006**	3	36	0.44	0.724
class*water	9	139	0.91	0.518	9	103	0.84	0.584

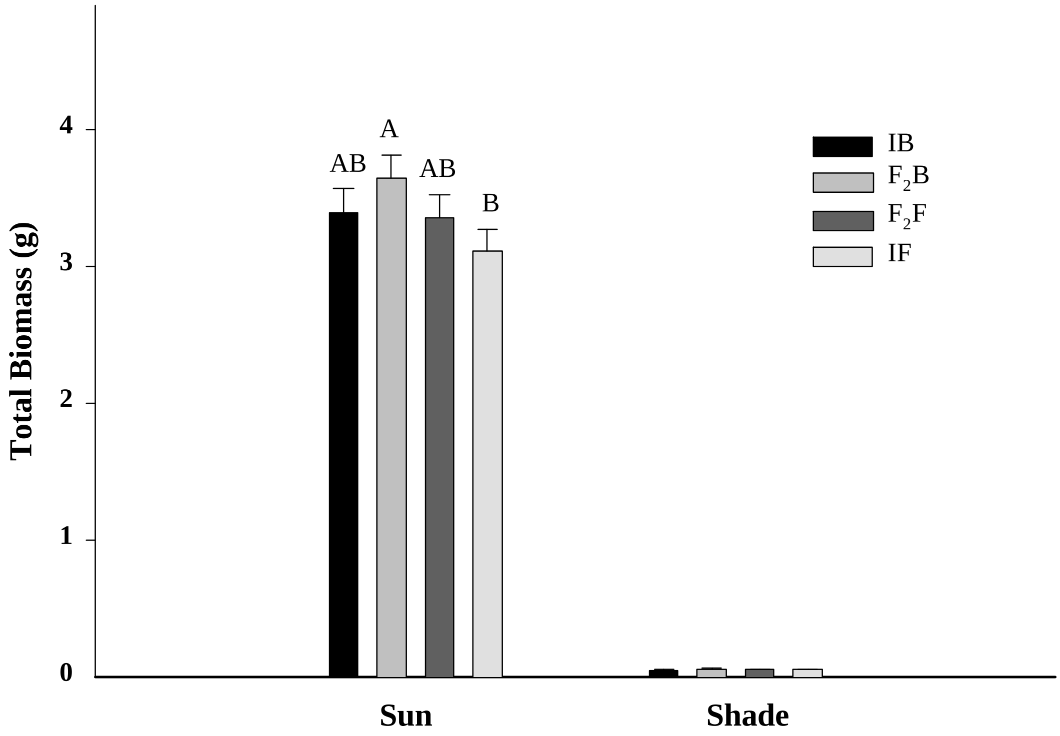

Figure 5.5. Total biomass of seedlings in four genotypic classes (*Iris brevicaulis* – IB, *I. fulva* – IF, and two F_2 hybrid crosses) of Louisiana Iris in experimental sun and shade treatments. The shade treatment represents a light reduction of 95%. Letters above bars indicate statistical relationships between classes within light treatments. There were no statistically significant differences among genotypic classes in the shade treatment

Table 5.5. Summary of relative fitness of four genotypic classes of Louisiana Iris (*I. brevicaulis*, *I. fulva*, and two F_2 hybrid crosses) at three early life stages. Seeds and seedlings were exposed to experimental light and water treatments to investigate environment dependence of hybrid fitness.

Life Stage	Environment	*I. brevicaulis*	F_2B hybrids	F_2F hybrids	*I. fulva*
Seed Germination	Sun	1.00	0.75	1.00	0.75
	Shade	0.34	0.79	0.76	1.00
Seedling Survival	Dry	0.53	1.00	0.66	0.78
	Field Capacity	0.55	0.92	0.92	1.00
	Flooded	0.68	1.00	0.92	0.96
Seedling Growth	Dry	0.85	1.00	0.89	0.86
	Field Capacity	0.97	1.00	0.98	0.99
	Flooded	0.95	1.00	0.92	0.67

DISCUSSION

Early life stage fitness components of these *Iris* genotypic classes responded very strongly to environmental condition. Because the distributions of adult *Iris* species and hybrids are associated with water and light gradients (Cruzan and Arnold 1993; Johnston et al. 2001a), we predicted that these genotypic classes would have different regeneration niches. Rather than a diversity of regeneration niches, species and hybrids responded to the same environmental cues, and appear to have similar requirements for germination and seedling success. Seeds only germinated in the driest of three water treatments, suggesting that seeds may require an oxidized environment to break dormancy (Ernst 1990). However, if seeds germinate in an environment that is dry, many seedlings may subsequently die (see Figure 5.3). The different needs of seeds and seedlings create an ecological seed-seedling conflict (Schupp 1995). The “ideal” regeneration niche must therefore be dynamic. Additionally, similar requirements for all genotypic classes may lead to competition between seedlings, which has not yet been tested empirically.

Hybrids do not, on average, have lower relative fitness at early life stages than *I. brevicaulis* or *I. fulva* (Table 5.5). Thus, hybrid seeds and seedlings are equally likely to pass through environmental sieves as individuals from *I. brevicaulis* or *I. fulva*. In several cases, the F_2B hybrid class is actually more fit than either parental species (Table 5.5). Burke et al. (1998) found similar high levels of germination in F_1 hybrids between *I. fulva* and *I. hexagona*, another Louisiana *Iris*. In the present study, seed germination does appear to be the most restrictive point in the life cycle of these *Iris* genotypic classes (14% germinated). Even slight differences in this fitness component will leave a deep mark on the resulting populations. Seeds that had not germinated at the end of the experiment were almost universally still viable, suggesting that seeds may remain dormant until conditions are favorable. However, there is no way of knowing from this experiment how long seeds can remain dormant in the wild. Pathogens and seed predators may take their toll on the seed bank, allowing only the fastest germinators to join the ranks of the population.

Seedling mortality overall was 16%. No mortality was measured in experiments with similar environmental manipulations of adult plants (Johnston et al. 2001a), suggesting that seedlings are more vulnerable than adult plants for both hybrid and parental individuals. Seedling growth rate is an important trait that moves seedlings out of the relatively dangerous pre-rhizome stage. Once plants establish some resources stored in the form of rhizome, like many clonal plants, a given genet may be virtually immortal (Gardner and Mangel 1999). Individual rhizomes (ramets) are able to recover from extreme shifts in abiotic conditions (*e.g.*. the drought of 2000 – Johnston, *unpublished data*), but seedlings seem more responsive to their abiotic environment (as in Figure 5.3).

Obviously *Iris* seeds and seedlings are vulnerable to their abiotic environment at early life stages, but in similar ways. Wetland environments are very dynamic in general (Mitsch and Gosselink 1993), and conflicting environmental requirements for seed germination and seedling survival to adulthood may be the best way to survive in a fluctuating environment. The narrow regeneration niche and the apparent potential for *Iris* seeds to remain viable during extended dormancy may work together to cause episodic recruitment in favorable years. Similar seed germination requirements have been found in other hybrid zones, but were followed by environment-dependent selection against seedlings (Wang et al. 1997, Campbell and Waser 2001). Grace and Wetzel (1982) report that *Typha latifolia* and *T. angustifolia* (both wetland species) germinate just at the edge of standing water, despite flood tolerance and colonization of standing water by both species at later life stages. Since irises are clonal, they are able to adjust their position within habitat patches after they have established. Rhizomes are 10-20 cm in length (Viosca 1935), allowing a plant to "walk" up or down slope, perhaps cuing in on favorable environmental conditions (Bazzaz 1991), resulting in ecological zonation of adult irises (Cruzan and Arnold 1993 Johnston et al. 2001a).

Previous studies have suggested that *I. brevicaulis* and *I. fulva* have different strategies of clonal and sexual reproductive allocation (Johnston, Donovan, and Arnold, *unpublished data*), and seed and seedling data support this idea. *I. brevicaulis* produces more, larger rhizomes on average, and fewer flowers than *I. fulva* in one experiment. With the lowest seed germination and seedling fitness of any genotypic class (Figure 5.2, Table 5.5), *I. brevicaulis* maximizes fitness by allocating more resources to rhizomes than flowers. *Iris fulva* adult plants are often small in terms of biomass (Table 5.6), but produce many flowers and have vigorous seeds and seedlings. Paradoxically, *I. brevicaulis* seems to be a more likely maternal parent of hybrids between *I. brevicaulis* and *I. fulva*. Cruzan and Arnold (1994) found that *I. brevicaulis* is more receptive to heterospecific pollen than *I. fulva*. There are not any backcrossed seeds in this experiment, but if hybrids were more likely to introgress toward *I. brevicaulis,* they may suffer from reduced fitness if they inherit the apparent seedling stage weaknesses of this species.

Table 5.6. Summary of relative fitness of *Iris brevicaulis, I. fulva* and first generation backcross hybrids. Values were calculated by comparing total biomass for each genotypic class within each environment.

Environment	*I. brevicaulis*	Backcross hybrids toward *I. brevicaulis*	Backcross hybrids toward *I. fulva*	*I. fulva*
dry[1]	0.99	0.90	0.98	1.00
field capacity[1]	1.00	0.90	0.92	0.92
waterlogged[2]	0.60	1.00	0.86	0.65
flooded[2]	0.66	1.00	0.86	0.65
sun[1]	1.00	0.90	0.90	0.92
shade[1]	1.00	0.91	0.99	0.99

[1] from Johnston et al. In prep(a)

[2] from Johnston et al. In prep(b)

However, in tests of environment-dependent fitness of early generation hybrids, the *I. brevicaulis*-like hybrids are consistently the more vigorous hybrid group (Table 4.6). The first generation backcrossed hybrids toward *I. brevicaulis* exhibits unique and extreme ecological traits and high fitness relative to parents and other hybrids (Johnston, Donovan, and Arnold, *unpublished data*). Overall, relative viability fitness of many hybrid genotypes is high in several abiotic environments. In many ways, hybrids between *I. brevicaulis* and *I. fulva* appear to inherit the best characters from both parents. Burke et al. (1998) drew a similar conclusion regarding hybrids between another Louisiana *Iris* species pair. In that study, F_1 hybrids between *I. fulva* and *I. hexagona* grew larger, produced more ramets, had higher pollen viability, and sired more seeds (Burke et al. 1998). Further studies of *I. brevicaulis* by *I. fulva* hybrids will include reciprocal transplants of seeds and rhizomes and fitness measures of *I. fulva, I. brevicaulis,* and hybrids in the field. Differences between experimental tests and field experiments may suggest biotic factors or life history stages whose role in hybrid fitness has not been yet considered. For now, we conclude that Louisiana *Iris* hybrids have high relative fitness at many life stages and in many environments.

ACKNOWLEDGEMENTS

The authors would like to acknowledge several people who made these studies possible. First and foremost, we want to thank C. Patterson for setting up and collecting data for the seed germination experiment as part of his REU project. Also, we are grateful to J. West and S. Dewalt for their technical expertise and advice. We express our gratitude to A. Tull and M. Boyd for watching over our plants and protecting them from experimental error. Several people helped with the harvest, including A. Bouck, L. Comita, S. Cornman, J. Espeleta, S. Franks, C. Richards, D. Rosenthal, C. Smith, J. West, and K. Snyder, who washed more roots than anyone. Many thanks to E. Kentner, S. Cornman, and E. Bouck for reviewing an earlier version of the manuscript. This research was supported by NSF-DEB-0074159 to MLA, an REU supplement from grant DEB-9703853, the UGA Office of the Vice President for Research, and American Iris Society and National Council of State Garden Clubs scholarships to JAJ.

LITERATURE CITED

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378-388.

Arnold, M. L. 1992. Natural Hybridization as an Evolutionary Process. Annual Review of Ecology and Systematics 23:237-261.

Arnold, M. L. 1994. Natural Hybridization and Louisiana Irises. Bioscience 44:141-147.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Arnold, M. L. 2000. Anderson's paradigm: Louisiana Irises and the study of evolutionary phenomena. Molecular Ecology 9:1687-1698.

Arnold, M. L., and S. A. Hodges. 1995. Are Natural Hybrids Fit or Unfit Relative to Their Parents. Trends in Ecology & Evolution 10:67-71.

Bazzaz, F. A. 1991. Habitat Selection in Plants. American Naturalist 137:S116-S130.

Bennett, B. D., and J. B. Grace. 1990. Shade Tolerance and Its Effect on the Segregation of 2 Species of Louisiana Iris and Their Hybrids. American Journal of Botany 77:100-107.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the Fitness of Hybrids. Annual Review of Genetics 35:31-52.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998. Hybrid fitness in the Louisiana irises: Analysis of parental and F-1 performance. Evolution 52:37-43.

Campbell, D. R., and N. M. Waser. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669-676.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and Genetic Associations in an Iris Hybrid Zone. Evolution 47:1432-1445.

Cruzan, M. B., and M. L. Arnold. 1994. Assortative mating and natural selection in an *Iris* hybrid zone. Evolution 48:1946-1958.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. The American Naturalist 74:312-321.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America 97:7043-7050.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Sciences of the United States of America 93:5090-5093.

Emms, S. K., and M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness: Transplant experiments with Louisiana irises. Evolution 51:1112-1119.

Ernst, W. H. O. 1990. Ecophysiology of Plants in Waterlogged and Flooded Environments. Aquatic Botany 38:73-90.

Gardner, S. N., and M. Mangel. 1999. Modeling investments in seeds, clonal offspring, and translocation in a clonal plant. Ecology 80:1202-1220.

Grant, B. R., and P. R. Grant. 1996. High survival of Darwin's finch hybrids: Effects of beak morphology and diets. Ecology 77:500-509.

Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. Science 256:193-197.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Grubb, P. J. 1977. The maintenance of species richness in plant communities. The importance of regeneration niche. Biological Reviews 52

Harper, J. L., J. T. Williams, and G. R. Sagar. 1965. The behavior of seeds in soil I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. Journal of Ecology 53:273-286.

Hobbs, R. J., and H. A. Mooney. 1985. Community and population dynamics of serpentine grassland annuals in relation to gopher disturbance. Oecologia 55:281-284.

Johnston, J. A., D. J. Grise, L. A. Donovan, and M. L. Arnold. 2001a. Environment-dependent performance and fitness of Iris brevicaulis, I-fulva (Iridaceae), and hybrids. American Journal of Botany 88:933-938.

Johnston, J. A., R. A. Wesselingh, A. C. Bouck, L. A. Donovan, and M. L. Arnold. 2001b. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana Iris hybrid population. Molecular Ecology 10:673-681.

Kindiger, B. 1994. A method to enchance germination of Eastern gamagrass. Maydica 39:53-56.

Kuuluvainen, T., and P. Juntunen. 1998. Seedling establishment in relation to microhabitat variation in a windthrow gap in a boreal Pinus sylvestris forest. Journal of Vegetation Science 9:551-562.

Martens, S. N., D. D. Breshears, and F. J. Barnes. 2001. Development of species dominance along an elevational gradient: Population dynamics of Pinus edulis and Juniperus monosperma. International Journal of Plant Sciences 162:777-783.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Mitsch, W. J., and J. G. Gosselink. 1993. Wetlands. John Wiley and Sons, New York, NY, USA.

Noe, G. B., and J. B. Zedler. 2001. Spatio-temporal variation of salt marsh seedling establishment in relation to the abiotic and biotic environment. Journal of Vegetation Science 12:61-74.

Orians, C. M. 2000. The effects of hybridization in plants on secondary chemistry: Implications for the ecology and evolution of plant-herbivore interactions. American Journal of Botany 87:1749-1756.

Rieseberg, L. H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rosenthal, D. M., A. E. Schwarzbach, L. A. Donovan, O. Raymond, and L. H. Rieseberg. In Press. Phenotypic differentiation between three anient hybrid taxa and their parental species. International Journal of Plant Sciences 00:000-000.

Schupp, E. W. 1995. Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. American Journal of Botany 82:399-409.

Stoltz, L. P. 1968. Iris seed dormance. Physiologia Plantarum 21:1328-1331.

Viosca, P. 1935. The irises of Southeastern Louisiana: a taxanomic and ecological interpretation. Bulletin of the American Iris Society 57:3-56.

Wagner, W. H., Jr. 1970. The role and taxonomic treatment of hybrids. Bioscience 19:785-795.

Wang, H., E. D. McArthur, S. C. Sanderson, J. H. Graham, and D. C. Freeman. 1997. Narrow hybrid zone between two subspecies of big sagebrush (Artemesia tridentata: asteraceae). IV. Reciprocal transplant experiments. Evolution 51:95-102.

Wesselingh, R. A., and M. L. Arnold. 2000. Nectar production in Louisiana Iris hybrids. International Journal of Plant Sciences 161:245-251.

CHAPTER 6

HIGH HYBRID FITNESS WITH LOW ENVIRONMENT-DEPENDENCE IN LOUISIANA IRIS RECIPROCAL TRANSPLANT EXPERIMENTS[1]

---

[1] Johnston, J. A., R. A. Wesselingh, J. T. Vogel, L. A. Donovan, and M. L. Arnold. To be submitted to *Evolution*.

ABSTRACT

Several models of hybrid zone evolution have been proposed to explain the maintenance of hybrid zones, and the importance of environmental context. The bounded hybrid superiority model proposes that hybrid genotypes can have higher fitness than their parent species, but only in habitat that is ecologically intermediate to parental habitats. Earlier greenhouse studies with Louisiana irises suggest that hybrid genotypes can be more fit than parental species in some experimental environments, but it is not clear how data from a controlled setting compare to hybrid zones in the wild. In the present study we measured viability fitness from adult and early life stages, and both vegetative and sexual reproduction in *Iris brevicaulis*, *I. fulva* and several early generation hybrid genotypic classes for three years in field conditions. Seeds and rhizomes were transplanted at four sites in Louisiana, each one home to natural populations of *I. brevicaulis*, *I. fulva*, or hybrid genotypes. We find evidence that early generation hybrids were more fit than the parent species in terms of adult survival, clonal reproduction and flowering. Seed germination did not differ across genotypic classes and years. Overall, seed and seedling fitness varied more with transplant site than genetic make-up. Hybrid fitness was higher at all sites, giving no indication that high hybrid fitness is restricted to intermediate habitat. Thus, our data do not support the predictions of bounded hybrid superiority.

INTRODUCTION

Variation in hybrid fitness in natural populations has recently received a great deal of attention from evolutionary biologists (Arnold and Hodges 1995, Arnold et al. 2001, Burke and Arnold 2001, Rieseberg et al. 2000, Barton 2001). Like any other population, hybrids swarms often possess considerable phenotypic variation (Rieseberg et al. 1999) which interacts with environmental conditions to form ecological associations (Cruzan and Arnold 1993, Johnston et al. 2001). If two parent species are uniquely adapted to different habitat types, hybrids can act as a bridge, allowing adaptive gene combinations to move between species, via introgression (Anderson 1949; Arnold 2000). Alternatively, genetic recombination during hybridization can generate new extreme or transgressive traits (de Vicente and Tanksley 1993; Rieseberg et al. 1999) which may increase hybrid fitness or be introgressed into one parent species or another. In some cases, natural hybrids are more fit in one specific habitat than in others, which may drive evolutionary divergence (Arnold 1993; Rieseberg 1997). In extreme cases, hybrids become ecologically aggressive, invading new habitat (Abbott 1992; Ellstrand and Schierenbeck 2000) or displacing one parent from its habitat (Anttila et al. 2000). For hybridization to act as a source or vector of adaptive variation, hybrid genotypes must have relatively high fitness in some environmental contexts (Anderson and Stebbins 1954; Arnold 1992; Barton and Hewitt 1985; Burke and Arnold 2001; Grant 1981).

A variety of theoretical models have been proposed to describe potential hybrid fitness and its effect on hybrid zone evolution (reviewed in Arnold 1997, Hewitt 1988). Each model approaches the relationship between hybrid fitness and environmental factors differently. Many of the proposed mechanisms that generate hybrid zone structure can result in the same spatial distribution of hybrid and parental genotypes (Arnold 1997; Barton and Hewitt 1985). It is difficult, therefore, to empirically test which mechanism is at work in any particular hybrid zone (Moore and Price 1993). However, some assumptions of individual models can be explicitly tested. One such hypothesis arises from the bounded hybrid superiority model. The bounded hybrid superiority model allows hybrids to be equally or even more fit than their parent species in environments that are intermediate to the habitats that parents are adapted to (Moore 1977). However, one assumption of the model is that hybrids will always be less fit than parental genotypes in parental habitat, thus limiting the success of hybrids to a narrow set of environmental conditions. If hybrids are more fit than parental species in their home habitat, the bounded hybrid superiority model can be ruled out. The classic test of adaptation to a specific type of habitat is the reciprocal transplant (Clausen et al. 1940). By measuring fitness of hybrid genotypes in their natural habitat, we get the best estimates of potential hybrid contribution to successive generations and whether or not that contribution is contingent on a specific environmental context (Graham et al. 2001; Hatfield and Schluter 1999; Wang et al. 1997).

Hybrid traits and their environmental associations have been investigated in the Louisiana iris species complex in several ways over the last fifty years (Anderson 1949; Bennett and Grace 1990; Emms and Arnold 1997). Two of these species, *Iris brevicaulis* Walter and *I. fulva* Ker-Gawler (IRIDACEAE) are both obligate wetland species, occurring along the edges of bayous and swamps throughout their ranges (Viosca 1935). The two species differ slightly in the microhabitat that they inhabit, with *Iris brevicaulis* living just above mean water line in slightly drier, sunnier habitat patches than *I. fulva* (Johnston et al. 2001, Cruzan and Arnold 1993). In southern Louisiana, hybrid zones can form where the two species co-occur (Arnold 1994). A series of greenhouse experiments were carried out to assess both the potential for hybrids to possess novel phenotypic traits and the extent to which hybrid fitness was environment dependent (Johnston, Arnold, and Donovan, *unpublished data*). Under greenhouse conditions, hybrids grew larger and produced more vegetative ramets than *I. brevicaulis* and *I. fulva*, in wet and flooded environments (Johnston, *unpublished data*). To fully understand the complexities of hybrid genotype by environment interactions, it is necessary to evaluate fitness of hybrids and their parent species under a range of realistic field conditions. In this study we were interested in two specific questions. First, do species and hybrids have higher relative fitness in their "home" habitat? And second, does relative fitness of each genotypic class differ between early life stages and adult stage under natural conditions? To address these questions, we performed reciprocal transplants of *I. brevicaulis*, *I. fulva*, and early generation hybrids, and measured fitness components over three years in the field.

METHODS

Species and hybrid genotypic classes

Several genotypic classes of early generation hybrids were used in this study. All hybrids were the product of researcher-assisted pollination in the University of Georgia Botany Department greenhouses in Athens, GA, USA. Rhizomes from both species were initially collected from natural populations in 1994 (*I. brevicaulis* - Assumption Parish, *I. fulva* – Terrebonne Parish, both in Louisiana, U.S.A.). Hereafter the two species will be referred to as IB and IF. Collected rhizomes were planted in standard potting mix and allowed to grow in well-watered and regularly fertilized conditions in the greenhouse.

For rhizome material, crosses between IF and IF were initiated in 1995 to produce F_1 hybrids. Both IF and IB served as the maternal parent of some seeds. The F_1 seeds were planted in the greenhouse in fall 1995. In spring1996, pollen from F_1 flowers was placed back onto IB and IF stigmas to produce first generation backcrosses toward IB, (BCIB) and IF, (BCIF). Seeds were again planted in the greenhouse, and by fall 1998 rhizome material was available for IB, IF, and BCIB, BCIF and F_1 hybrid classes.

Seed material was obtained from crosses made in spring 1998. IB and IF seeds were made by crossing bulked pollen onto conspecific stigmas. Two classes of F_1 hybrid seeds were made, with IB or IF serving as maternal parent (F_1(bxf) or F_1(fxb)). Transferring F_1 pollen onto stigmas of other F_1 hybrid individuals that shared maternal parentage generated two F_2 hybrid classes, F_2(bxf) and F_2(fxb), with IB or IF maternal parents, respectively.

Study sites

Four sites were used in the reciprocal transplant experiments, three in St. Martin Parish, and one in Terrebonne Parish, Louisiana (Figure 1). Each site had a different mixture of wild irises prior to the experiment. The Babineaux (BAB) site contained only individuals of IB. The Foti Forest (FFO) and Foti Pasture (FPA) sites contained a mixture of both IB and IF, and a variety of hybrid genotypes. Earlier sampling at these sites indicated that hybrid genotypes at FPA have backcrossed with IB, and hybrids at FFO have backcrossed with IF (Cruzan and Arnold 1993). The Talbot (TAL) site (in Terrebonne Parish) contained only wild IF plants (Hodges et al. 1996). Although site conditions were not extensively characterized during the course of this study, previous work at these locations suggest that BAB is the driest, FFO and FPA have intermediate soil moisture, and TAL is considerably wetter (Cruzan and Arnold 1993; Emms and Arnold 1997; Hodges et al. 1996).

Seed germination experiment

To study the effects of genotypic class and site on germination, seeds from IB, IF, F_1(fxb), F_1(bxf), F_2(fxb), and F_2(bxf) were planted in 15 replicate blocks at each site. Eight rows of 10 seeds were planted in a grid, spaced 10 cm apart. Each block contained 20 seeds of IB and IF, and 10 seeds of each hybrid class. Blocks were at least 0.5 m apart. Each seed was marked with a wooden tag so that it could be relocated after several months. Seedlings were censused for germination in spring 1999 and germination and survival in spring 2000. Plots at 3 sites were obscured by disturbance during the summer of 2000, making a 2001 seedling census impractical.

Rhizome transplant experiment

Survival, and production of ramets and flowers was measured on a cohort of plants derived from rhizome transplants. Five genotypic classes were used, IB, IF, BCIB, BCIF, and F_1 hybrids. Ten, one meter-square blocks of 25 rhizomes (5 of each genotypic class) were planted at each site. Each rhizome was held in place by a gardening staple 15 cm in length. A total of 600 rhizomes were transplanted in September 1998, 150 at each site. These consist of approximately 30 rhizomes from each genotypic class at each site.

Plants growing from transplanted rhizomes were censused every spring and fall during the course of the experiment. Survival and number of shoots were recorded at each census date. Every shoot produces a rhizome that becomes an independent ramet the following year. Shoots production therefore represents vegetative reproduction (Wikberg 1995; Wikberg et al. 1994). Flowers and flower stalks were noted in the spring. Since generally only one fruit forms on each flower stalk produced (Wesselingh and Arnold, *unpublished data*), we present the number of flower stalks as our best estimate of reproductive fitness. In May 2001, plants were censused for the last time.

Statistical analyses

Germination, seedling survival and rhizome data were converted into proportions on a per block scale. Proportions were arcsine square root transformed (Sokal and Rohlf 1995) before analysis with ANOVA in PROC GLM in SAS (SAS institute, Cary, NC). Number of shoots produced was analyzed as an ANCOVA with initial rhizome weight as covariate. Contrasts were used to perform post hoc means comparisons following all analyses. Flower stalks were so few that the data were not statistically tractable. Raw numbers of flower stalks are presented.

RESULTS

Seedling germination and survival

A total of 462 seeds (9.6%) germinated in our experimental plots. In both 1999 and 2000, there were significant differences in the proportion of seeds that germinated at the four sites, but no differences among the six genotypic classes (IB, IF, F_1(fxb), F_1(bxf), F_2(fxb), and F_2(bxf), Table 6.1). Each year, an average of 29 seeds germinated (out of 600 planted) in each hybrid class, and about 58 seeds germinated (out of 1200) from each species. Germination at each site varied between years (Figure 6.1). When data from both years are combined, it is clear that more seeds germinate in FPA than any other site, BAB and FFO are intermediate, and TAL has the lowest germination. Seedling survival, however, were also significantly different among sites ($p = 0.002$, $df_{3,23}$), but not among genotypic classes. At 86% survival, more seedlings survived for one year at

Table 6.1. ANOVA results from a seed germination experiment designed test hybrid fitness in several natural environments. Two species, *Iris brevicaulis* and *I. fulva* were used in the experiment, as well as seeds from four types of hybrid cross between them. Seeds were planted at four sites, representing the habitat of both species and hybrids. A total of 120 seeds (out of 4800) germinated over two years.

Germination		1999		2000	
Source	df	F	p	F	p
genotypic class	5	1.98	0.0814	0.61	0.6931
site	3	4.79	0.0028	17.32	<.0001
class*site	15	0.67	0.8095	0.83	0.6464
error	336				

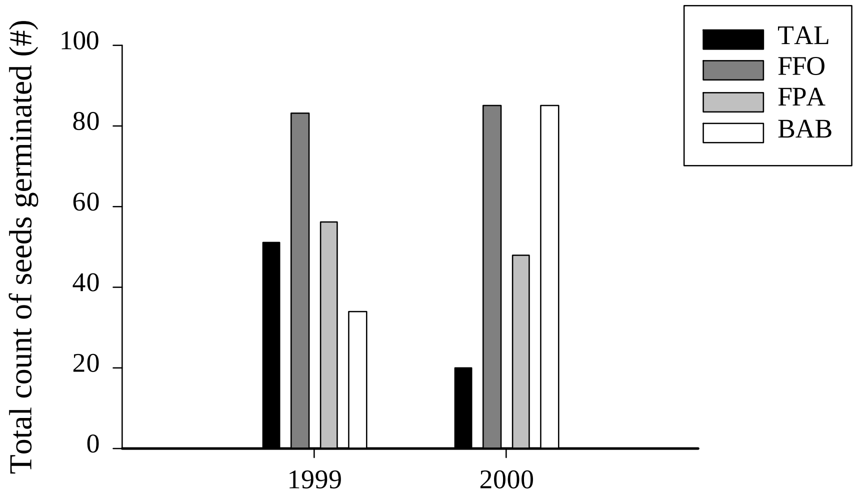

Figure 6.1. Louisiana iris seed germination in 1999 and 2000. Seeds from six genotypic classes, two species and four types of hybrid cross, were planted at four sites in Louisiana. The proportion of seeds that germinated did not differ among genotypic classes, so the pooled data of all genotypic classes are presented here to demonstrate site differences.

TAL than at any other site. Seedlings at the two hybrid sites survived at rates of 54% and 49%, and 29% of seedlings survived their first year at BAB.

Rhizome transplant experiment

Over the course of the experiment 390 (65%) of plants derived from transplanted rhizomes died. Survival patterns were different among classes and sites, but there was not a significant interaction between the two (Table 6.2). Means comparisons show that F_1 plants survived better than all other genotypic classes when averaged across sites. Among sites, FFO had the highest survival (42%), BAB and FPA were intermediate (34% and 33%), and TAL had the lowest survival (28%) of transplanted rhizomes. The overall shape of the survivorship curves is different among sites, but genotypic classes within sites generally behave in a similar manner (Figure 6.2).

The number of shoots produced each year by surviving plants was influenced by different factors in different years. Shoots produced in 1999 were affected by the covariate, initial rhizome mass, and by site (Table 6.3, Figure 6.3). In 2000 there were significant main effects of initial rhizome mass, genotypic class and site. The plants in TAL clearly produce more shoots than the other sites, and the F_1 and BCIB hybrids produced more shoots than other genotypic classes (Figure 6.3). By the third growing season (2001), there was no longer an effect of initial rhizome mass, and both class and site main effects remain significant. Differences among the classes were more pronounced by the final measurement, and the F_1 hybrids have produced more shoots per plant than other genotypic classes (Figure 6.3). The interaction between site and class was not significant in any of the three years (Table 6.3).

Table 6.2. Results of ANOVA on survival of Louisiana Iris rhizomes planted into experimental gardens at four sites. Five genotypic classes were used, *Iris brevicaulis, I. fulva*, F_1 hybrids, and first generation backcrosses toward each parent. Each site represents natural habitat of either species or hybrid populations. 65% of adult plants died during the three year study period.

Survival		1999		2000		2001	
Source	df	F	p	F	p	F	p
genotypic class	4	2.82	0.031	5.09	0.001	4.86	0.002
site	3	22.1	<0.001	25.9	<0.001	2.15	0.102
class*site	12	1.12	0.358	1.45	0.164	1.1	0.371
error	70						

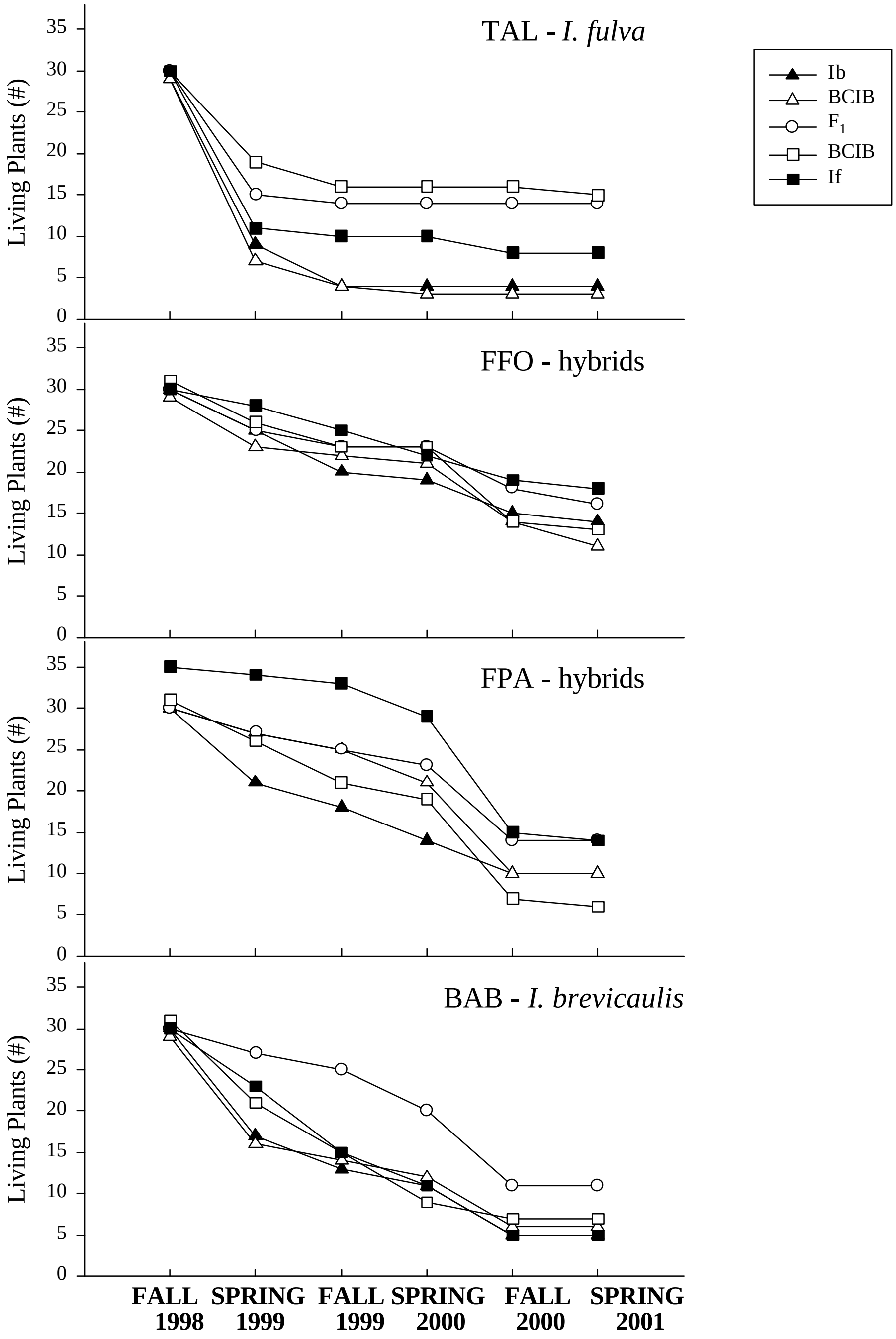

Figure 6.2. Survival among transplanted rhizomes from five genotypic classes of Louisiana iris at four sites. Two species (*Iris brevicaulis* - Ib and *I. fulva* - If) and three hybrid crosses (F_1, and first generation backcrosses – BCIB and BCIF) were used in this study. Each site has a native iris population of *I. brevicaulis* – BO, *I. fulva* – TB, and hybrids – FF and FP. Survival was monitored for three growing seasons.

Table 6.3. Results from ANOVA on number of vegetative ramets produced by five genotypic classes of Louisiana iris (*Iris brevicaulis*, *I. fulva*, F_1 hybrids, and first generation backcrosses toward each species) planted at four sites in Louisiana. Fresh weight of rhizomes planted in fall 1998 is the covariate

Shoots	1999			2000			2001		
Source	df	F	p	df	F	p	df	F	p
covariate	1	75.4	<0.001	1	17.8	<0.001	1	0.01	0.925
genotypic class	4	1.74	0.140	4	4.36	0.002	4	4.58	0.002
site	3	3.98	0.008	3	6.56	<0.001	3	25.8	<0.001
class*site	12	1.67	0.072	12	1.74	0.058	12	1.55	0.112
error	402			288			167		

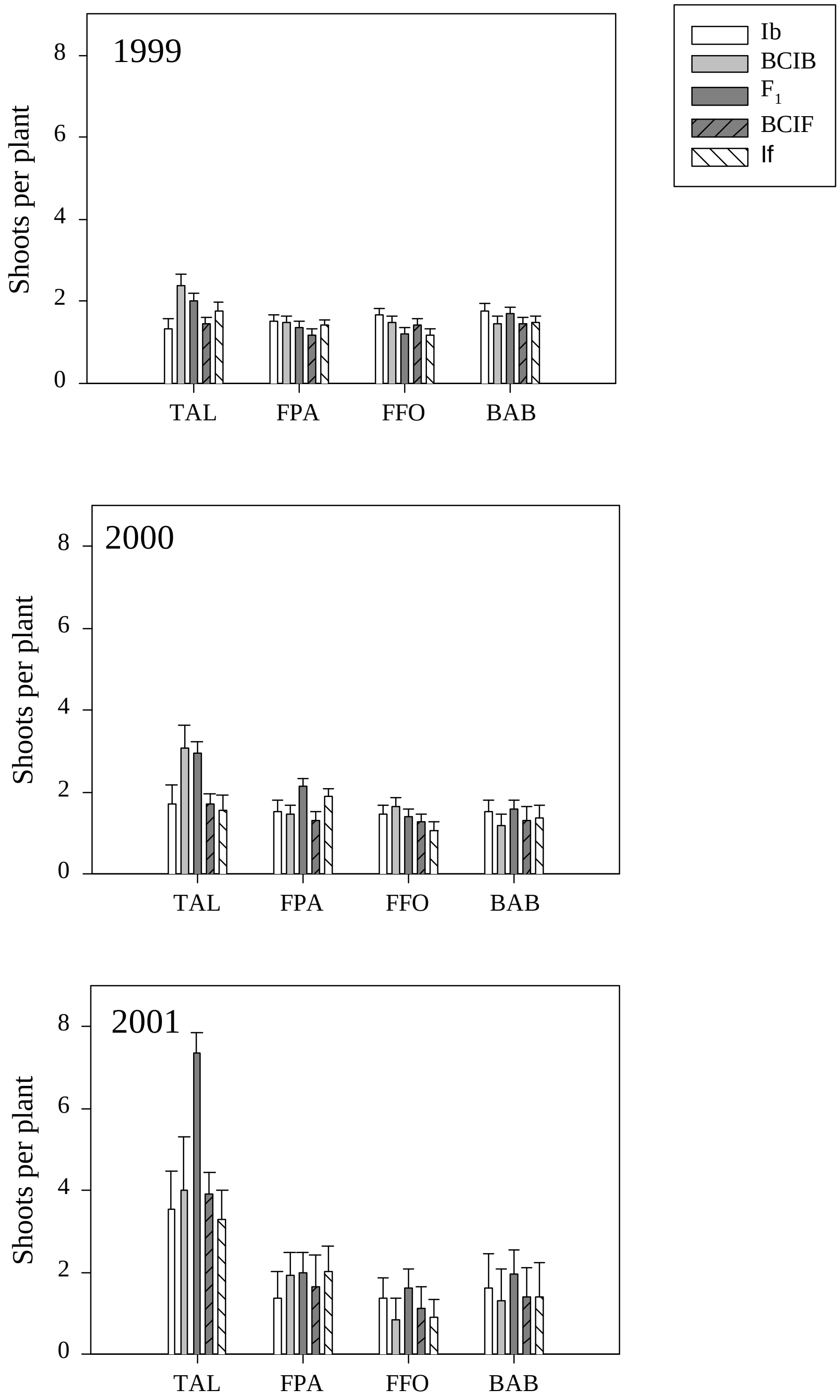

Figure 6.3. Average number of shoots, or vegetative ramets, produced by five genotypic classes of Louisiana iris (*I. brevicaulis* – Ib, *I. fulva* –If, F$_1$ hybrids between them, and first generation backcrosses toward each parent – BCIB and BCIF) at four sites that each has a native iris population (BAB - *I. brevicaulis*, FFO and FPA – hybrids, and TAL – *I. fulva*).

Since only a small number of plants flowered during the course of the experiment, flower data were not statistically analyzed. A few plants flowered the first two years at both FFO and FPA, (Table 6.4). In 2001, TAL was the only site where plants flowered, and no plants ever flowered at BAB (Table 6.4). All classes flowered at some point during the experiment (Figure 6.4), but IB only produced flowers in 2000 (Table 6.4). The most flowers were produced by two hybrid groups, F_1 and BCIF (Table 6.4, Figure 6.4).

DISCUSSION

Hybrid fitness and environmental context

The bounded hybrid superiority model predicts that hybrids have high relative fitness compared to parent species only in intermediate, or hybrid habitat. The hybrid fitness patterns that we measured do not fit the assumptions of the bounded hybrid superiority model (Moore 1977). Neither do they support the tension zone (Barton and Hewitt 1985) or mosaic model (Harrison 1986), both of which predict hybrid fitness will always be lower than parents in nature. Our findings do not support the assumptions of the bounded hybrid superiority model, because these Louisiana Iris hybrids have high fitness in all environments tested. Not only were hybrids superior across environments, but performance rank of all genotypic classes for any given fitness component was relatively consistent across sites and no genotypic class by site interactions were significant (see Tables 2, 3). While genotypic class differences in ramet production and survival were small at some sites, when one genotypic class is clearly the most fit, it is a hybrid group (e.g. Figure 3, 4). Hybrids also demonstrated high fitness in greenhouse experiments (Johnston, Donovan, and Arnold, *unpublished data*), but such high average

Table 6.4. Number of flower stalks produced by five genotypic classes of Louisiana Iris at four sites, over three years. Data presented are raw count data. Genotypic classes are: *Iris brevicaulis*, *I. fulva*, their F_1 hybrids, and first generation backcrosses toward each species. The sites are each inhabited by either one species (BO – *I. brevicaulis*, TB – *I. fulva*) or natural hybrids (FF – *I. brevicaulis*-like hybrids, FP – *I. fulva*-like hybrids).

Flower stalks	1999						2000						2001					
Site	If	Bf	F_1	Bb	Ib	total	If	Bf	F_1	Bb	Ib	total	If	Bf	F_1	Bb	Ib	total
BAB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FFO	-	1	2	-	-	3	-	2	3	-	-	5	-	-	-	-	-	-
FPA	3	1	2	-	-	6	1	2	5	-	1	9	-	-	-	-	-	-
TAL	-	-	1	-	-	1	2	4	7	1	1	13	1	13	21	1	-	24
total	3	2	5	-	-	10	3	8	15	1	2	27	1	13	21	1	-	24

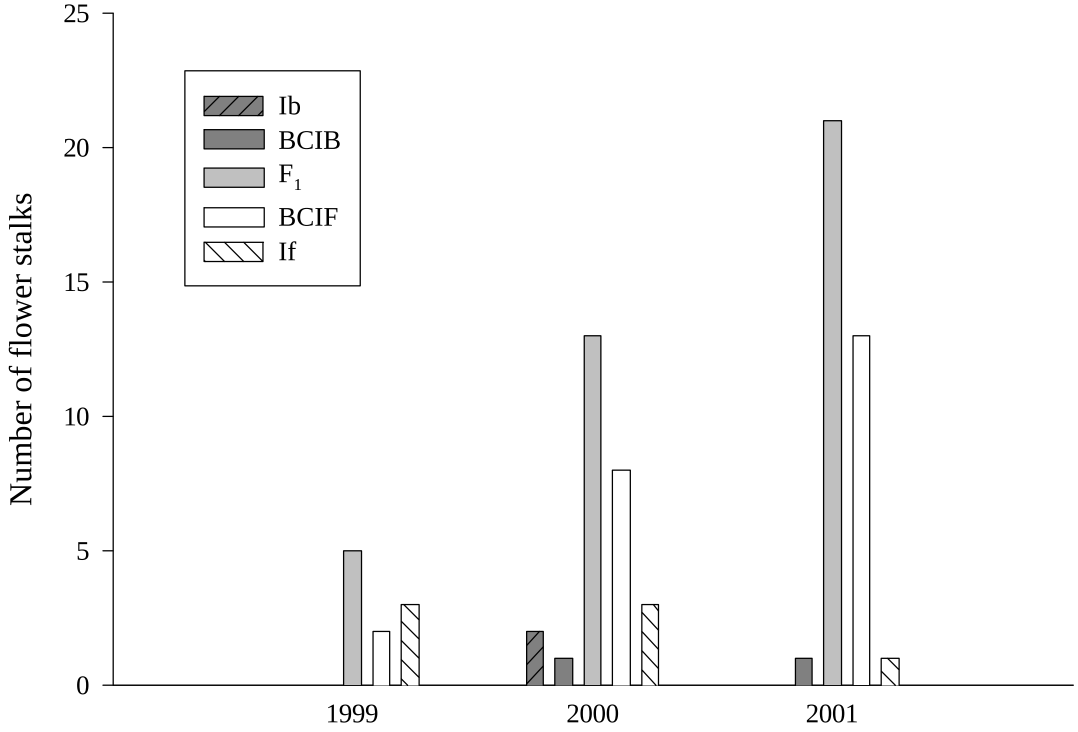

Figure 6.4. Number of flower stalks produced by each genotypic class each year. Numbers here are shown pooled among sites. Number of flowers produced per stalk was variable, with an overall average of 4.5. Because on average one fruit forms per stalk regardless of species identity, number of flower stalks was chosen as the most easily comparable unit of reproductive fitness.

hybrid performance in terms of survival as well as clonal and sexual reproductive output is surprising in a natural setting. While many studies find that hybrids have are capable of achieving high fitness in a subset of natural conditions (Campbell and Waser 2001; Emms and Arnold 1997; Graham et al. 1995), previous studies commonly found reduced fitness when experiments are moved from the lab or greenhouse to the field (Hatfield and Schluter 1999; Vamosi et al. 2000). Our results support the hybrid novelty model (Arnold 1997), which suggests some hybrids are not necessarily unfit in any environment. However, it is not clear from the present data whether genotype by environment interactions were causing high hybrid fitness, or if some other mechanism was at work.

As clonal plants, irises have many fitness components, including both clonal and sexual reproduction, survival from seed to seedling stage, accumulation of enough resources to form a rhizome (the transition to adult stage), and survival of adult ramets from one year to the next (Gardner and Mangel 1999; Wikberg 1995). It is expected that plants have limited access to resources which will necessarily lead to trade-offs between resource demanding processes such as clonal vs. sexual reproduction (Mendez and Obeso 1993; Wijesinghe and Whigham 1997; Worley and Harder 1996). The F_1 hybrids between these two species frequently appear to be capable of maximizing all measured fitness components at once, producing more flower stalks, more ramets, and surviving better than both parent species, simultaneously. Hybrid pollen has been shown to be very fertile, > 90% on average (Cruzan and Arnold 1994), so it is unlikely that hybrids are losing reproductive quality in exchange for producing a large quantity of reproductive organs. Both parent species have mixed mating systems and are believed to be widely outcrossing (Cruzan et al. 1994) which led us to predict that heterosis would play only a small role in early generation hybrid fitness. However, the magnitude of hybrid advantage and high average fitness of hybrids in all environments suggests that heterosis, may contribute to the observed pattern.

If hybrid fitness is so high in natural conditions, why are hybrids not taking over? Formation of F_1s appears to be the most restrictive reproductive step, and once hybridization begins, it is not clear what stops these two species from fusing. Backcross hybrids toward *I. fulva* exhibited high relative fitness in our transplant gardens, while backcrosses toward *I. brevicaulis* did not. Cruzan and Arnold (1994) found that *I. brevicaulis* had a more flexible genetic background for heterospecific genes, and that therefore backcrossing in natural hybrid zones was more likely to be toward *I. brevicaulis* than *I. fulva*. The fact that backcrossed hybrids toward *I. brevicaulis* are not as fit in a natural setting as backcrossed hybrids toward *I. fulva*, might mean that there is environmental selection opposing the genetic asymmetry of this particular hybridization. The conflicting selective pressures may constrain hybrid fitness over several generations.

There may be other factors that can limit hybrid fitness but were not present during the three years in which we performed the present study. Severe fluctuations in abiotic conditions often leave lasting impressions on wetland community composition (Howard and Mendelssohn 2000) and may exert strong, periodic selection (Hoffmann and Hercus 2000) that can affect hybrid fitness and structure hybrid zones (Grant and Grant 1996). There may be episodic biotic selection pressures that were not observed at these sites from 1998-2001. It is possible that F_1 hybrids are very prolific as pollen and seed parents, but if large numbers of either parent species are present, introgression toward that parent proceeds so quickly that it is difficult to catch recognizable hybrids in a natural hybrid population (Hodges et al. 1996). Alternatively, we may be observing a few genotypes that possess real hybrid novelty (*sensu* Arnold 1997) that are driving the differences in genotypic class means. For instance, an individual from a single rhizome transplant was responsible for six of the 21 flower stalks produced at the Talbot site (TAL) in 2001. Further analysis of individual plant performance will be explored in a future paper.

Habitat associations of parent species

Iris brevicaulis and *I. fulva* do not appear to be specifically adapted to their "home" habitats. While detailed site descriptions were not made during this study, differences in water table between the Talbot site and the other three sites were very apparent (Cruzan and Arnold 1993; Hodges et al. 1996). Nonetheless, neither species had especially high relative fitness at any of the sites, except at the *I. fulva*-like hybrid site, where *I. fulva* had the highest survival of any genotypic class (Figure 3). Between the two species, *I. fulva* had higher survival in all environments. The number of flower stalks and shoots produced by the two species was similar, counter to expectations based on greenhouse experiments. When the two species are in controlled conditions, *I. brevicaulis* allocates more to clonal reproduction and *I. fulva* makes a larger flowering effort (Johnston, Arnold, and Donovan, *unpublished data*). In the field, the two species appear to allocate their resources in a more similar manner. The two species are probably able to tolerate a large range of environmental conditions, and perhaps extreme events or dispersal limitations keep them from living in same type of habitat. We can measure habitat differences between the two species in established mixed populations (Cruzan and

Arnold 1993; Johnston et al. 2001), so presumably microsite environmental differences affect species distributions within site. It is possible that we have incorrectly perceived the relevant scale of habitat differences. Within each site, the range of habitats presented may encompass the optimal habitat of each species. Spatial analysis may reveal within-site environment dependence of fitness for *I. brevicaulis* and *I fulva*.

Early life stage vs. adult fitness components

Relative hybrid fitness at seed and seedling stages were quite different from hybrid fitness at the adult stage. No differences were detected in relative performance of the six genotypic classes when germination was pooled across all sites. Overall, seed germination was low, with 8-11% germinating in each genotypic class over two years. Louisiana irises germinate in the greenhouse at a rate of 75-87% in one year (Burke et al. 1998). There was no way to evaluate the fate of seeds that didn't germinate. Some of them may have been viable but dormant, but several of them were undoubtedly dead or eaten. Seedlings are rarely found in the field (Arnold 1994; Hodges et al. 1996), which may indicate that they are infrequent or short-lived. Greenhouse studies suggest that both species and early generation hybrids have similar germination requirements (Johnston, Arnold, and Donovan, *unpublished data*), which is a pattern repeated through many wetland plant communities (Grace and Wetzel 1982; Lorenzen et al. 2000). Seedling survival from 1999-2000 was 52% overall, but did not differ among genotypic classes. The seedling stage appears to be a very narrow selection bottleneck through which these irises must pass. Any differences in fitness at this stage could have a large impact on the adult population structure. However, our data indicate that seed germination and survival are not life stages at which hybrid genotypes are any more vulnerable to selection than their parental species.

Conclusions

Hybrids between *Iris brevicaulis* and *I. fulva* have high relative fitness under natural conditions, which defies the bounded hybrid superiority, tension zone, and mosaic models of hybrid zone evolution. Hybrid classes are very fit in a variety of environments. As a genotypic class, F_1 hybrids have the greatest advantage over parental genotypes, possibly due to heterosis, an especially benign environment during this study, or true hybrid novelty. Backcross hybrids toward *I. fulva*, also appear to have high fitness compared to the parent species, but its advantage is environment-dependent. *Iris brevicaulis* and *I. fulva* are not specifically adapted to the habitats in which they naturally occur. Seed germination and seed survival were low, and differed among sites but not among genotypic classes. Thus, selection on adult stages appears to be most important in structuring hybrid zones of these Louisiana irises.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the many people who made this study possible. First, we are eternally grateful to D. Bienvenu, S. Babineaux, W. Talbot, and the late F.C. Foti and family for allowing us to establish transplant plots on their property. Many people helped collect data in the field, including A. Bouck, S. Cornman, E. Kentner, J. West, A. Salmore, R. Klaper, J. Cruse, S. Madson, C. Patterson, R. Peeler, C. Richards, C. Connelly, and L. Johnston. We are indebted to S. Mopper, P. van Zandt, and M. Tobler for sharing their knowledge of ecology on the bayou. S. Franks, C. Richards, D. Rosenthal, and K. Snyder gave valuable feedback on earlier versions of this manuscript. Funding for this project came from NSF grant # DEB-00774519 to M.L.A., and small grants from the American Iris Society, the National Council of State Garden Clubs, and the UGA Botany Department to J.A.J.

LITERATURE CITED

Abbott, R. J. 1992. Plant Invasions, Interspecific Hybridization and the Evolution of New Plant Taxa. Trends in Ecology & Evolution 7:401-405.

Anderson, E. 1949. Introgressive hybridization. Wiley & Sons, New York.

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378-388.

Anttila, C. K., R. A. King, C. Ferris, D. R. Ayres, and D. R. Strong. 2000. Reciprocal hybrid formation of Spartina in San Francisco Bay. Molecular Ecology 9:765-770.

Arnold, M. L. 1992. Natural Hybridization as an Evolutionary Process. Annual Review of Ecology and Systematics 23:237-261.

Arnold, M. L. 1993. Iris-Nelsonii (Iridaceae) - Origin and Genetic Composition of a Homoploid Hybrid Species. American Journal of Botany 80:577-583.

Arnold, M. L. 1994. Natural Hybridization and Louisiana Irises. Bioscience 44:141-147.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Arnold, M. L. 2000. Anderson's paradigm: Louisiana Irises and the study of evolutionary phenomena. Molecular Ecology 9:1687-1698.

Arnold, M. L., and S. A. Hodges. 1995. Are Natural Hybrids Fit or Unfit Relative to Their Parents. Trends in Ecology & Evolution 10:67-71.

Arnold, M. L., E. K. Kentner, J. A. Johnston, S. Cornman, and A. C. Bouck. 2001. Natural hybridisation and fitness. Taxon 50:93-104.

Barton, N. H. 2001. The role of hybridization in evolution. Molecular Ecology 10:551-568.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16:113-148.

Bennett, B. D., and J. B. Grace. 1990. Shade Tolerance and Its Effect on the Segregation of 2 Species of Louisiana Iris and Their Hybrids. American Journal of Botany 77:100-107.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the Fitness of Hybrids. Annual Review of Genetics 35:31-52.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998. Hybrid fitness in the Louisiana irises: Analysis of parental and F-1 performance. Evolution 52:37-43.

Campbell, D. R., and N. M. Waser. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669-676.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1940. Experimental Studies on the Nature of Species. I. Carnegie Institution of Washington, Washington, D.C., USA.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and Genetic Associations in an Iris Hybrid Zone. Evolution 47:1432-1445.

Cruzan, M. B., and M. L. Arnold. 1994. Assortative mating and natural selection in an *Iris* hybrid zone. Evolution 48:1946-1958.

Cruzan, M. B., J. L. Hamrick, M. L. Arnold, and B. D. Bennett. 1994. Mating System Variation in Hybridizing Irises - Effects of Phenology and Floral Densities on Family Outcrossing Rates. Heredity 72:95-105.

de Vicente, M. C., and S. D. Tanksley. 1993. Qtl Analysis of Transgressive Segregation in an Interspecific Tomato Cross. Genetics 134:585-596.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America 97:7043-7050.

Emms, S. K., and M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness: Transplant experiments with Louisiana irises. Evolution 51:1112-1119.

Gardner, S. N., and M. Mangel. 1999. Modeling investments in seeds, clonal offspring, and translocation in a clonal plant. Ecology 80:1202-1220.

Grace, J. B., and R. G. Wetzel. 1982. Niche Differentiation between 2 Rhizomatous Plant-Species - Typha-Latifolia and Typha-Angustifolia. Canadian Journal of Botany-Revue Canadienne De Botanique 60:46-57.

Graham, J. H., D. C. Freeman, and E. D. McArthur. 1995. Narrow Hybrid Zone between 2 Subspecies of Big Sagebrush (Artemisia-Tridentata, Asteraceae) .2. Selection Gradients and Hybrid Fitness. American Journal of Botany 82:709-716.

Graham, J. H., E. D. McArthur, and D. C. Freeman. 2001. Narrow hybrid zone between two subspecies of big sagebrush (Artemisia tridentata : Asteraceae) - XII. Galls on sagebrush in a reciprocal transplant garden. Oecologia 126:239-246.

Grant, B. R., and P. R. Grant. 1996. High survival of Darwin's finch hybrids: Effects of beak morphology and diets. Ecology 77:500-509.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Harrison, R. G. 1986. Pattern and Process in a Narrow Hybrid Zone. Heredity 56:337-349.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution 53:866-873.

Hewitt, G. M. 1988. Hybrid Zones - Natural Laboratories for Evolutionary Studies. Trends in Ecology & Evolution 3:158-167.

Hodges, S. A., J. M. Burke, and M. L. Arnold. 1996. Natural formation of iris hybrids: Experimental evidence on the establishment of hybrid zones. Evolution 50:2504-2509.

Hoffmann, A. A., and M. J. Hercus. 2000. Environmental stress as an evolutionary force. Bioscience 50:217-226.

Howard, R. J., and I. A. Mendelssohn. 2000. Structure and composition of oligohaline marsh plant communities exposed to salinity pulses. Aquatic Botany 68:143-164.

Johnston, J. A., R. A. Wesselingh, A. C. Bouck, L. A. Donovan, and M. L. Arnold. 2001. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana Iris hybrid population. Molecular Ecology 10:673-681.

Lorenzen, B., H. Brix, K. L. McKee, I. A. Mendelssohn, and S. L. Miao. 2000. Seed germination of two Everglades species, Cladium jamaicense and Typha domingensis. Aquatic Botany 66:169-180.

Mendez, M., and J. R. Obeso. 1993. Size-Dependent Reproductive and Vegetative Allocation in Arum- Italicum (Araceae). Canadian Journal of Botany-Revue Canadienne De Botanique 71:309-314.

Moore, W. S. 1977. Evaluation of Narrow Hybrid Zones in Vertebrates. Quarterly Review of Biology 52:263-277.

Moore, W. S., and J. T. Price. 1993. Nature of selection in the northern flicker hybrid zone and its implications for speciation theory. Pp. 196-225 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford University Press, Oxford, UK.

Rieseberg, L. H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg, L. H., S. J. E. Baird, and K. A. Gardner. 2000. Hybridization, introgression, and linkage evolution. Plant Molecular Biology 42:205-224.

Sokal, R. R., and J. F. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., New York.

Vamosi, S. M., T. Hatfield, and D. Schluter. 2000. A test of ecological selection against young-of-the-year hybrids of sympatric sticklebacks. Journal of Fish Biology 57:109-121.

Viosca, P. 1935. The irises of Southeastern Louisiana: a taxanomic and ecological interpretation. Bulletin of the American Iris Society 57:3-56.

Wang, H., E. D. McArthur, S. C. Sanderson, J. H. Graham, and D. C. Freeman. 1997. Narrow hybrid zone between two subspecies of big sagebrush (Artemesia tridentata: asteraceae). IV. Reciprocal transplant experiments. Evolution 51:95-102.

Wijesinghe, D. K., and D. F. Whigham. 1997. Costs of producing clonal offspring and the effects of plant size on population dynamics of the woodland herb Uvularia perfoliata (Liliaceae). Journal of Ecology 85:907-919.

Wikberg, S. 1995. Fitness in Clonal Plants. Oikos 72:293-297.

Wikberg, S., B. M. Svensson, and B. A. Carlsson. 1994. Fitness, Population-Growth Rate and Flowering in Carex- Bigelowii, a Clonal Sedge. Oikos 70:57-64.

Worley, A. C., and L. D. Harder. 1996. Size-dependent resource allocation and costs of reproduction in Pinguicula vulgaris (Lentibulariaceae). Journal of Ecology 84:195-206.

CHAPTER 7

CONCLUSIONS

ECOLOGICAL DIFFERENCES BETWEEN PARENT SPECIES

Habitats occupied by *Iris brevicaulis* and *I. fulva* in a natural population where significantly different where they co-occurred. On average, *I. fulva* was found in a wetter, shadier habitat than *I. brevicaulis*. *Iris brevicaulis* existed in a wide range of possible habitats, while *I. fulva* occupied a narrow range of habitat, and appeared to be restricted to areas that remained saturated most of the time. Greenhouse experiments did not support the predictions generated by these field observations, namely that *I. brevicaulis* was more drought tolerant, and *I. fulva* was more shade and flood tolerant. However, in the reciprocal transplant experiment in the field, *I. fulva* did have higher fitness in flooded areas than *I. brevicaulis*. It seems likely that *I. fulva* is better adapted to a biotic factor such as a pathogen that is associated with a flooded environment, and not flooding itself. Alternatively, the difference in habitat occupied may be due to a factor that cannot be evaluated with plants grown in standard pots, such as rooting depth. Overall, field data support the habitat differences along a water gradient for *I. brevicaulis* and *I. fulva*, but there is little evidence to support differences in shade tolerance.

EVIDENCE OF HYBRID NOVELTY

Evidence of extreme trait expression in hybrids between *I. brevicaulis* and *I. fulva* was most obvious in the fitness components measured. Both positive and negative extreme expression of physiological and fitness related characters were seen in hybrids at both the genotypic class and individual genotype levels. These results imply that the process of hybridization itself can generate novel phenotypic variation in physiological and ecological traits. In many ways, hybrids between *I. brevicaulis* and *I. fulva* appear to inherit the best characters from both parents. The F_1 hybrids between these two species frequently appear to be capable of maximizing all measured fitness components at once, producing more flower stalks, more ramets, and surviving better than both parent species, simultaneously. While the most extreme expression of hybrid traits was seen in the F_1 generation, back crossed hybrids toward *I. brevicaulis* had extreme expression of fitness components in the greenhouse, while back crossed hybrids toward *I. fulva* had extremely high expression of fitness components in the field. It is possible that the physiological traits affected most by hybridization were not measured in this study, but hybrid fitness was clearly very high in a large range of environments.

ENVIRONMENTAL DEPENDENCE OF HYBRID FITNESS

There were several types of evidence that showed hybrid fitness was generally high and strongly dependent on environmental conditions. Relative hybrid fitness was more environmentally dependent at the adult stage. Hybrid fitness at seed and seedling stages were very subject to the environment but in a similar way to both parent species. When individual hybrid genotypes were examined, some of the most fit individual genotypes in one environment were among the least fit in the other. Habitat distributions of natural hybrids were unique from parent species distributions, and the fact that genetic markers showed environmental association indicated that environment dependent selection was operating on hybrid genotypes in natural conditions. Specifically, field and greenhouse data both suggested that Louisiana Iris hybrids may have relatively high fitness in wet and sunny areas, but are likely to be inferior to their parents in the dry or shaded conditions. Thus, habitat that is heterogeneous for light at a fine scale, like that found along the bayous of southern Louisiana may promote hybrid success.

Hybrids between *Iris brevicaulis* and *I. fulva* exhibited high relative fitness under several types of natural conditions, including habitat of the two parent species. The hybrid fitness patterns that were measured in reciprocal transplants do not fit the assumptions of the bounded hybrid superiority model (Moore 1977), the tension zone (Barton and Hewitt 1985) or mosaic model (Harrison 1986). While many studies find that hybrids are capable of achieving high fitness in a lab or greenhouse setting (Hatfield and Schluter 1999; Vamosi et al. 2000a), hybrid fitness is often reduced in natural conditions (Campbell and Waser 2001; Emms and Arnold 1997; Graham et al. 1995). Our results support the predictions of the hybrid novelty model (Arnold 1997), which assumes that some hybrids may have high fitness in one or more environments. However, it is not clear from the present data whether genotype by environment interactions were causing high hybrid fitness in our experimental tests, or if some other mechanism underlies the pattern.

DISCREPANCIES BETWEEN FIELD AND GREENHOUSE

Differences between experimental tests and field experiments suggest that perhaps selection by biotic factors or selection at unstudied life history stages may play a large role in hybrid fitness. Based on the findings of these studies, the author concludes that Louisiana *Iris* hybrids have high relative fitness at many life stages and in many environments. Formation of F_1's appears to be the most restrictive reproductive step, and once hybridization begins, it is not clear that viability fitness and genotype by environment interactions will stop these two species from fusing. Backcross hybrids toward *I. fulva* exhibited high relative fitness in our transplant gardens. Backcrosses toward *I. brevicaulis* exhibited high fitness in greenhouse tests, but low fitness in field experiments. There may be environmental factors capable of limiting hybrid fitness that were not present during the three years in which the reciprocal transplant study was performed. Severe fluctuations in abiotic conditions often leave lasting impressions on wetland community composition (Howard and Mendelssohn 2000) and may exert strong, periodic selection (Hoffmann and Hercus 2000) that can affect hybrid fitness and structure hybrid zones (Grant and Grant 1996). There may be episodic biotic selection pressures that was weak during the three year field study, and was completely absent in the greenhouse. It is possible that F_1 hybrids are very fit in terms of sexual reproduction, but if large numbers of either parent species are present, backcrossing toward that parent proceeds so quickly that hybrids have little chance to proliferate in natural populations (Hodges et al. 1996). Alternatively, there may have been a few genotypes in these studies that possessed real hybrid novelty (*sensu* Arnold 1997) that are driving the differences in genotypic class means, and observed high hybrid fitness. Hybridization between these two species has the potential to generate extensive variation, some of which affects physiological traits and has increases the habitat range that these two species can otherwise occupy. Thus, it appears hybridization is an important source of adaptive genetic variation in this species complex.

LITERATURE CITED

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16:113-148.

Campbell, D. R., and N. M. Waser. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669-676.

Emms, S. K., and M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness: Transplant experiments with Louisiana irises. Evolution 51:1112-1119.

Graham, J. H., D. C. Freeman, and E. D. McArthur. 1995. Narrow Hybrid Zone between 2 Subspecies of Big Sagebrush (Artemisia-Tridentata, Asteraceae) .2. Selection Gradients and Hybrid Fitness. American Journal of Botany 82:709-716.

Grant, B. R., and P. R. Grant. 1996. High survival of Darwin's finch hybrids: Effects of beak morphology and diets. Ecology 77:500-509.

Harrison, R. G. 1986. Pattern and Process in a Narrow Hybrid Zone. Heredity 56:337-349.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution 53:866-873.

Hodges, S. A., J. M. Burke, and M. L. Arnold. 1996. Natural formation of iris hybrids: Experimental evidence on the establishment of hybrid zones. Evolution 50:2504-2509.

Hoffmann, A. A., and M. J. Hercus. 2000. Environmental stress as an evolutionary force. Bioscience 50:217-226.

Howard, R. J., and I. A. Mendelssohn. 2000. Structure and composition of oligohaline marsh plant communities exposed to salinity pulses. Aquatic Botany 68:143-164.

Moore, W. S. 1977. Evaluation of Narrow Hybrid Zones in Vertebrates. Quarterly Review of Biology 52:263-277.

Vamosi, S. M., T. Hatfield, and D. Schluter. 2000. A test of ecological selection against young-of-the-year hybrids of sympatric sticklebacks. Journal of Fish Biology 57:109-121.