

High hybrid fitness at seed and seedling life history stages in Louisiana irises

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Summary

1 Relative fitness of hybrid genotypes will determine the role that hybrids can play in the evolution of a plant species complex. To realistically evaluate hybrid fitness and how environmental variation affects fitness in a long-lived species, all life stages must be considered.

2 We evaluated germination, seedling survival and growth of two Louisiana iris species and their early generation hybrids in several experimental environments created by manipulating light and water levels.

3 Species and hybrids all required similar moist conditions for germination. The proportion of germinated seeds was highest in shade and seedling survival highest in sun.

4 *Iris brevicaulis* exhibited the lowest germination and seedling survival overall, yet those individuals that survived grew vigorously. *Iris fulva* had high levels of germination and seedling survival, but yielded the smallest plants at the end of one season of growth.

5 Germination rates, seedling survival and seedling growth of hybrids equalled or exceeded one or both parent species, indicating that hybrids in this system have high relative fitness at seed and seedling stages in several environmental conditions.

6 We conclude that *Iris fulva* and *I. brevicaulis* share a common regeneration niche and similar early stage relative fitness with their hybrids. Thus, environment-dependent fitness in this system is due primarily to selection at adult stages.

Key-words: environment-dependent selection, germination, hybridization, hybrid zone, seedling survival

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Introduction

Hybridization is gaining credibility as a creative evolutionary force in wild plant populations (Anderson & Stebbins 1954; Arnold 1997; Rieseberg 1997), following several decades of scepticism 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 & Grace 1990; Grant & Grant 1992; Orians 2000). Some hybrid genotypes will inevitably have low fitness due to genetic complications, but hybrid fitness is usually highly variable (Cruzan & Arnold 1994; Rosenthal *et al.* 2002) and often includes some relatively fit genotypes with the potential to contribute new genetic variation to

future generations (Grant & Grant 1996; Emms & Arnold 1997; Burke & Arnold 2001).

Environmental context and life history traits are often important components of hybrid success (Arnold & Hodges 1995; Rieseberg *et al.* 1999). Many plant species can tolerate a large range of environmental conditions at the adult stage, while requiring a specific narrow range of conditions for successful seed germination and seedling establishment (Grubb 1977; Hobbs & Mooney 1985). Closely related species can have very different requirements for germination (Harper *et al.* 1965), which can distinguish species that are ecologically similar as adults (Grubb 1977). When different ecological selection factors act on the regeneration niche compared with adult plants, selection at later stages will alter patterns begun by spatially patchy germination (Martens *et al.* 2001; Noe & Zedler 2001). If germination requirements are specific (Harper *et al.* 1965), and seedling establishment is restricted to a narrow range of abiotic factors (Grubb 1977; Kuuluvainen & Juntunen 1998), genetic changes that affect characteristics of the 'regeneration

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niche' could drive ecological divergence of populations, even when adult individuals are ecologically equivalent.

In long-lived or clonally reproducing taxa, there are many life stages upon which selection can act, making it difficult to predict regeneration niche patterns from the distribution of adult plants (Grace & Wetzel 1982; Martens *et al.* 2001). Many successful plant hybrids occur in long-lived or clonally reproducing taxa (Ellstrand *et al.* 1996). When hybrids occur among annual plants, selection on germinating seeds and seedlings can have a very immediate effect on the population structure and ecological distribution patterns (Wolf *et al.* 2001). Even with multiyear studies, it is difficult to evaluate all components of fitness for long-lived hybrids. Many studies use clonal replicates and measure adult fitness traits or look at adult distribution patterns (Weber & D'Antonio 1999; Johnston *et al.* 2001a). While this is useful, little is known about perennial hybrids and their regeneration niches or fitness at early life stages.

Adult viability and reproductive fitness components have been extensively studied in Louisiana irises, a group of naturally hybridizing, perennial, 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. Hybrid genotypes can produce flowers that combine the long life span of *I. fulva* and the high nectar concentration of *I. brevicaulis* (Wesselingh & Arnold 2000), resulting in attractive targets for pollinators. Hybrid pollen is successful at fertilizing parent species and generating viable backcrossed offspring (Cruzan & Arnold 1994). Survival and performance are generally high for adult Louisiana iris hybrids, but relative hybrid fitness changes with environmental condition such as drought or deep shade (Johnston 2002). While F_1 's are rare in natural populations due to a cascade of reproductive barriers between species, once a hybrid genotype becomes established, it can act as a bridge that facilitates further gene movement between species via introgression (Arnold 2000). As hybrids backcross towards parents, beneficial traits that arose during hybridization can be integrated into the genetic background of either parent species, potentially increasing fitness for the entire population (Anderson & Stebbins 1954; Arnold 1992; Ellstrand & 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 early life 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, seedling growth and survival? Secondly, does relative hybrid fitness at seed and seedling stages change across environmental conditions?

Methods

STUDY SPECIES BIOLOGY

Iris brevicaulis and *I. fulva* are obligate wetland plants, occurring along the edges of bayous and swamps throughout their ranges (Viosca 1935). Water and light levels are the primary and secondary abiotic factors that distinguish the habitats of the two species (Johnston *et al.* 2001b). *Iris brevicaulis* lives just above the mean water line in slightly drier, sunnier habitat patches than *I. fulva* (Cruzan & Arnold 1993; Johnston *et al.* 2001b). In southern Louisiana, these iris species occur in sparsely shaded patches that are often waterlogged but rarely flooded for long periods of time (Cruzan & Arnold 1993). Hybrid zones can form in these areas (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 crosses. Hybrid crosses were initiated in 1997 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. Pollen was transferred within pools of F_1 hybrids (one group with IB and one with IF cytoplasm) in 1998 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 . F_1 seeds were not available for use in this experiment. All seeds used in this experiment were produced during the 1998 growing season.

The germination experiment was carried out in the Botany Department greenhouses at the University of Georgia, Athens, GA, USA. Light and water conditions were experimentally manipulated and the effect of each environmental treatment combination on seed germination was measured for all four genotypic classes described above. Seeds were planted in pots with nine 5 cm³ compartments, which allowed the manipulation of environmental conditions on eight seeds at a time (leaving one randomly chosen compartment empty). Pots were filled with potting soil, and seeds were buried just below the soil surface. There were three light treatments: sun (100% ambient), shade (50% ambient) and deep shade (25% ambient), similar to those used in a previous shading study with Louisiana irises (Bennett & Grace 1990). Light treatment levels were achieved by stretching neutral shade cloth on a PVC frame that was suspended a few centimetres above the soil surface. Three water treatments were used: moist (watered but well drained), flooded (submerged) and fluctuating (alternating between flooded and moist). All plants were watered two times per week for the duration of the experiment. Each nine-compartment pot was placed in a plastic tub designed to impose one of the three water

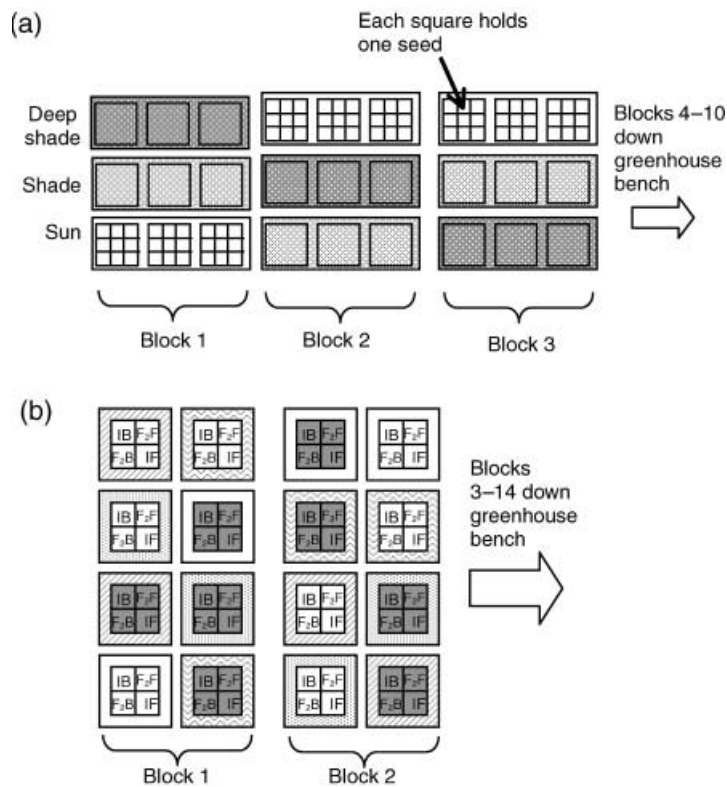


Fig. 1 (a) Experimental set-up of seed germination. For the germination experiment, light treatment split plots (deep shade, shade, sun) are represented by filled or open rectangles, and each large square represents a nine-hole flat insert that is placed in its own water treatment tray (moist, flood, fluctuating). The whole experimental unit or block was replicated 10 times on a glasshouse bench. To set up the seedling growth and survival experiment (b), four pots containing one seedling of each type were independently assigned a water treatment (dry, moist, flooding, and fluctuating, represented by fill pattern), and sun or shade treatment (open or dark inner square). The block was replicated 14 times on a glasshouse bench.

treatments. Moist treatment tubs had holes drilled in them 1 cm from their bottoms. Flooded tubs had no holes and the soil surface was kept submerged under 1 cm of water. Fluctuating treatments involved switching pots between plastic tubs with and without holes every 4 weeks.

With four genotypic classes (IB, F₂B, F₂F, IF), three light treatments (sun, shade, deep shade), three water treatments (moist, flooded, fluctuating), there were 36 treatment combinations in this experiment. The experiment was set up in a split-split plot design. The split plot factor was light, and the split-split plot factor was water. Each treatment combination was replicated two times per experimental unit or block, and the block was replicated 10 times on a glasshouse bench (Fig. 1a), for a total of 720 seeds. Germination was monitored and recorded twice weekly from 15 May 1999 to 15 December 1999. Seven months represented the approximate time interval of one growing season, and germination had tapered off at the time of harvest. Plants were removed from the experimental array as they germinated 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.

A total of 99 seeds germinated out of 720 planted, all in the moist (watered, but well-drained) treatment.

Germination data from the moist treatment were analysed for effects of genotypic class and light. Germination proportions for seeds in each treatment combination were calculated for each block. Proportions were arcsine square root transformed and analysed in SAS PROC MIXED, which uses maximum likelihood procedures to explicitly model random and fixed treatment effects and interactions resulting in different output than sums of squares procedures. Post hoc means comparisons were performed with independent contrasts.

SEEDLING GROWTH EXPERIMENT

An experiment to measure growth and survival of seedlings was conducted using additional seeds from the crosses described above. Iris seeds may take several months to germinate unassisted (Burke *et al.* 1998). To facilitate the study of same-aged seedlings, seed scarification began on 31 October 2000 to synchronize germination. Seeds were soaked in water for 24 h, then in 4% hydrogen peroxide for 30 min (Kindiger 1994) before scarification. Seeds were then removed from their corky outer covering. The seed coat was cut with a razor blade, and a small piece of endosperm removed to expose the embryo (Stoltz 1968). Twenty scarified seeds were placed on 130 mm Petri plates containing *c.* 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 min, then poured.) Plates were sealed and left on a laboratory bench receiving indirect, natural light for 1 week. When both root and shoot were visible, seeds were removed from plates and placed in individual cups containing 10 mL of fresh agar. Cups were sealed in plastic deli containers to prevent desiccation. Three weeks after scarification, all available seedlings were transplanted into 5 cm³ 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. While we recognize that manipulating seeds and seedlings may involve selection, these were necessary steps for following a cohort of seedlings. It is possible that we inadvertently selected for 'early' germination genotypes, but the handling was consistent across all plants, and should not have affected comparisons among genotypic classes.

Light and water were manipulated in the seedling growth experiment. Four levels of water were used: dry, moist, flooded, and fluctuating. All plants were watered with a drip tube system that provided 1 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, moist 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 alternating plastic containers every 4 weeks. Two levels of light were used, sun and shade. Sun plants were given full ambient light, and shade plants were placed under small cloth tents. The light level in the shade treatment was about 10% ambient, similar to levels expected on the ground in a fully leafed out hardwood forest (Hutchinson & Matt 1977). Experimental treatments began on 15 December 2000.

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 subplot factor (Fig. 1b). Light and water treatment combinations were assigned at random to sets of four seedlings, containing one each from IB, F₂B, F₂F and IF. With four levels of genotypic class, four levels of water and two levels of light, there were 32 treatment combinations in all. Each combination was present once within each experimental unit and there were 14 replicates of the experiment across a glasshouse bench.

During the course of the experiment, leaf area was measured three times at 7-week intervals (15 December – treatment initiation; 2 February and 27 March – 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 check 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.

Growth and total biomass were analysed with PROC MIXED in SAS with block as the random effect. Raw growth data violated the assumption of normality, and were square root transformed before analysis. Growth was analysed as a split-plot repeated measures ANOVA with first order autoregressive correlation structure. Total biomass was analysed as split-plot ANOVA in PROC MIXED.

Seventy seedlings died during the course of the experiment, prompting an evaluation of seedling mortality. Because the experiment was not initially designed to measure mortality, pairs of experimental replicates were grouped to form artificial blocks. Proportional mortality (number dead/total in each 'block') was analysed using an ANOVA in PROC MIXED, after proportions were arcsine square root transformed. Post hoc comparisons were performed with independent contrasts.

To summarize relative hybrid fitness, relative fitness of each genotypic class was calculated in each environment tested (Silvertown & Lovett Doust 1993, p. 2). This effectively summarizes the results from multiple experiments and life stages, allowing for performance comparisons among environments. The calculation used LSMEANS generated by SAS PROC MIXED. Genotypic classes were ranked by their LSMEAN for each fitness component (seed germination, seedling survival, seedling biomass) for each environmental treatment, then divided by the largest LSMEAN. In this way, there was always one genotypic class that had a fitness of one, and all other classes had a fitness in that environment that was some fraction of one.

Results

SEED GERMINATION EXPERIMENT

Seed germination was greatly influenced by environmental conditions, but demonstrated similar patterns across genotypic classes. All seeds that germinated were in the moist watering treatment; only those data were analysed further. Germination was also strongly influenced by light environment and genotypic class (Table 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 1), so average germination is shown for each class in each light treatment (Fig. 2). In the shade treatments more seeds germinated from IF than from IB. Both hybrid classes tended to be intermediate (Fig. 2).

Table 1 Results of an ANOVA on proportion of seeds that germinated in each of four genotypic classes of Louisiana irises. *Iris brevicaulis*, *I. fulva* and two types of F₂ hybrids were tested for germination in full sun, shade, and deep shade. Effects that are significant below the *P* = 0.05 level are in bold, ndf = numerator degrees of freedom, ddf = denominator degrees of freedom, class = genotypic class

Source	ndf	ddf	<i>F</i>	<i>P</i>
Class	3	81	4.21	0.008
Light	2	18	14.3	< 0.001
Class × light	6	81	1.97	0.079

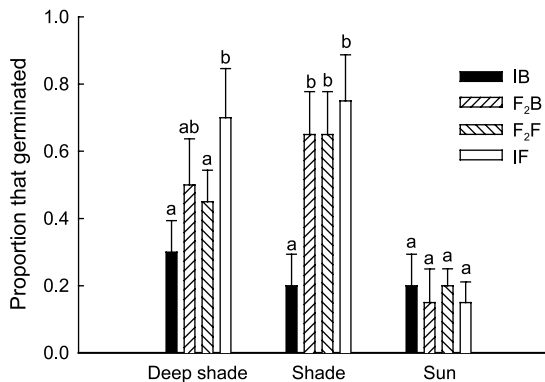


Fig. 2 Proportion of seeds that germinated in four genotypic classes of Louisiana iris (IB, *I. brevicaulis*, IF, *I. fulva*, and two F₂ hybrid crosses). All data shown were from the moist water treatment, separated by light treatments. Letters indicate results from means comparisons within each light treatment.

Table 2 Results of an ANOVA on the proportional mortality in 444 Louisiana iris seedlings grown for one season in a glasshouse. Four genotypic classes, *I. brevicaulis*, *I. fulva*, and two F₂ hybrid crosses were subjected to combinations of experimental shade and water treatments and monitored for one growing season. Seventy seedlings died during the experiment. Effects that are significant below the *P* = 0.05 level are shown in bold, ndf = numerator degrees of freedom, ddf = denominator degrees of freedom, class = genotypic class

Source	ndf	ddf	<i>F</i>	<i>P</i>
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

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

SEEDLING GROWTH AND SURVIVAL

In the seedling growth and survival experiment, 70 out of 448 seedlings died. Four seedlings died due to equipment failure, and were eliminated from the analyses. Genotypic class, water and light all had significant main effects on mortality (Table 2). While there were

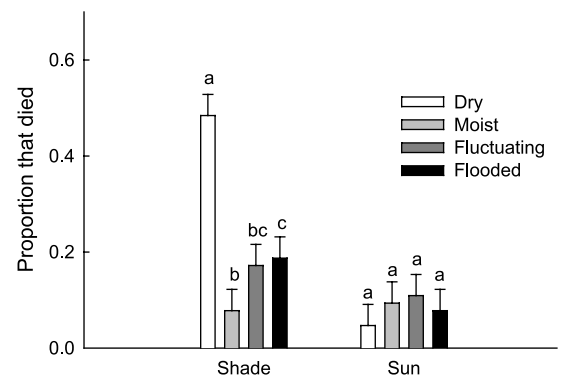


Fig. 3 Mortality among seedlings of two species and two hybrid crosses of Louisiana irises during one season of growth in a glasshouse. Proportions of plants that died are shown grouped by light treatments, with one bar representing each water treatment used in the experiment. Letters above bars denote significant differences among water treatments within each light treatment.

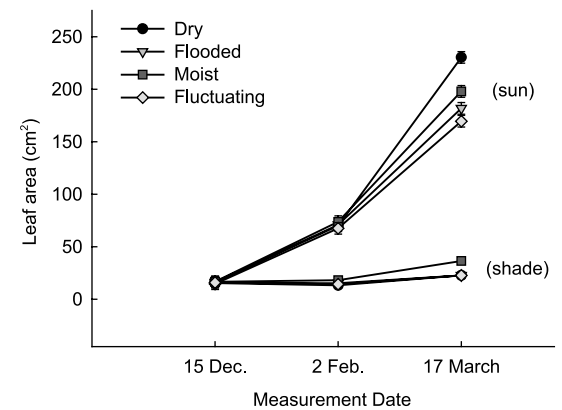


Fig. 4 Seedling growth curves of two Louisiana irises and their second-generation hybrids in four water treatments, dry, moist, flooded, and fluctuating, and two light treatments, sun and shade. The top set of curves represents plants grown in the sun and the lower cluster of curves shows growth of plants in the shade treatment. In the sun, plants growing in dry conditions grew significantly faster than those in flooded and fluctuating water conditions. Error bars on the shade growth curves are smaller than the symbols used in the figure.

not any interactions between genotypic class and either environmental factor, means comparisons revealed that a greater proportion of IB seedlings died than all other genotypic classes (LSMEAN proportional mortality IB = 27%, F₂B = 8%, F₂F = 13%, IF = 14%). High mortality in all genotypic classes in the dry, shade treatment resulted in a significant interaction between water and light (Fig. 3).

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 analysed as separate data sets (Fig. 4). Estimated growth rates in sun plants differed among genotypic classes and water treatments (significant class × time, water × time, Table 3). The F₂B hybrid class grew to have the largest leaf area (LSMEAN ± SE = 14.0 ± 0.25 cm²), and was significantly larger than IF, the

Table 3 Results from a repeated measures ANOVA of Louisiana iris seedling leaf area during one growing season. *Iris brevicaulis*, *I. fulva*, and two types of F₂ hybrid cross were subjected to combinations of light and water treatment. Leaf area was measured three times during the experiment as a non-destructive estimate of growth. Sun and shade plants were analysed separately. Effects that are significant below the $P = 0.05$ level are in bold, ndf = numerator degrees of freedom, ddf = denominator degrees of freedom, class = genotypic class

Source	Sun				Shade			
	ndf	ddf	<i>F</i>	<i>P</i>	ndf	ddf	<i>F</i>	<i>P</i>
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 4 Results from ANOVA analysis of total biomass in Louisiana iris seedlings at the end of one growing season. *Iris brevicaulis*, *I. fulva*, and two types of F₂ hybrid cross were subjected to combinations of light and water treatments and grown in a glasshouse. The two light treatments were analysed separately due to extreme differences in seedling size in sun and shade. Effects that are significant below the $P = 0.05$ level are in bold, ndf = numerator degrees of freedom, ddf = denominator degrees of freedom, class = genotypic class

Total biomass Source	Sun				Shade			
	ndf	ddf	<i>F</i>	<i>P</i>	ndf	ddf	<i>F</i>	<i>P</i>
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

Table 5 Summary of relative fitness of four genotypic classes of Louisiana iris (*I. brevicaulis*, *I. fulva*, and two F₂ 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. Across each row in the table, all values for that fitness component and environment were divided by the largest value. Thus, a fitness of 1.0 was assigned to the best performer in the row, and a proportion to all other genotypic classes. No statistical significance is implied by these numbers

Life stage	Environment	<i>I. brevicaulis</i>	F ₂ B hybrids	F ₂ F hybrids	<i>I. fulva</i>
Seed germination	Sun	1.00	0.93	0.91	0.84
	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

smallest class ($13.3 \pm 0.25 \text{ cm}^2$, $P = 0.047$). Among watering treatments, the dry treatment plants grew the largest, and were significantly different from flooded and fluctuating treatments with the moist treatment intermediate (Fig. 4). Among shade plants, growth rate did not differ among genotypic classes (class × time not significant, Table 3). Water was the only factor that significantly affected growth rate, with moist plants growing significantly faster than all other groups (Fig. 4).

Both genotypic class and water treatment had significant effects on total biomass of plants in the sun treatment (Table 4). The F₂B genotypic class had the largest average biomass, and was significantly bigger than IF (Fig. 5), while IB and F₂F were intermediate.

Biomass patterns among watering treatments were similar to final leaf areas (dry > fluctuating = moist ≥ flooded). Total biomass of shaded plants was not significantly different among genotypic classes or water treatments (Table 4).

Overall, hybrids had high relative fitness for all early stage fitness components tested. Furthermore, relative hybrid fitness was similar in all environmental conditions examined for seeds and seedlings (Table 5).

Discussion

Hybrids in this experiment did not have a lower average relative fitness at early life stages than *I. brevicaulis* or

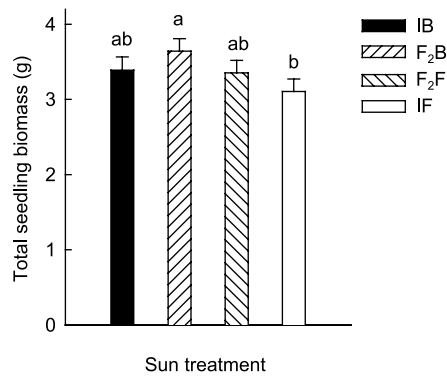


Fig. 5 Total biomass of seedlings in four genotypic classes (*Iris brevicaulis*, IB, *I. fulva*, IF, and two F₂ hybrid crosses) of Louisiana iris grown for one growing season in full sun. Letters above bars indicate statistical relationships between the average biomass attained in each genotypic class.

I. fulva. Thus, hybrid seeds and seedlings are equally likely to pass through environmental sieves as seeds and seedlings of *I. brevicaulis* or *I. fulva*. In several cases, the F₂B hybrid class is actually more fit than either parental species. Burke *et al.* (1998) found similar high levels of germination in F₁ hybrids between *I. fulva* and *I. hexagona*, another Louisiana iris. Seed germination and seedling survival appear to be restrictive points in the life cycle of an iris, considering adult plants had 100% survival in an earlier study with environmental manipulations (Johnston *et al.* 2001a). These results suggest that seedlings are more vulnerable to environmental conditions than both hybrid and parental species adult individuals. Seedling growth rate is an important trait that moves seedlings out of the relatively dangerous pre-rhizome stage. While there was some variation in growth rates among genotypic classes, this fitness component will matter most on the individual scale, not group averages. The plants with the fastest growth rates due to the interaction of genetics and environmental conditions will be out of danger first. Once plants establish some resources stored in a rhizome, like many clonal plants, a given genet may be virtually immortal (Gardner & Mangel 1999). Individual rhizomes are able to recover from extreme shifts in abiotic conditions (e.g. the drought of 2000; Johnston, unpublished data), but seedlings are more subject to environmental changes.

Early life stage fitness components of these Iris genotypic classes responded very strongly to the environment, but relative performance of hybrids did not. Because the distributions of adult iris species and hybrids are associated with water and light gradients (Cruzan & Arnold 1993; Johnston *et al.* 2001b), 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 all genotypic classes appear to have similar requirements for germination and seedling success. Seeds only germinated in the moist water treatment, suggesting that seeds may

require an aerobic environment to break dormancy (Ernst 1990). If seeds germinate in an environment that becomes too dry, many seedlings may subsequently die due to drought sensitivity. However, seedlings that survive in dry conditions seem to grow larger than plants in other water treatments, suggesting that there may be a fine line between stressful and optimal growing conditions for these young plants. The ideal regeneration niche for these iris species and hybrids is likely to be ephemeral and dynamic, occurring only when soil moisture and light fall within appropriate ranges.

Wetland environments are dynamic in general (Mitsch & Gosselink 1993), so specific germination requirements and the potential for iris seeds to remain viable during extended dormancy may work together to cause episodic recruitment in favourable years. In other hybrid systems, hybrids and parent species have been shown to have similar germination requirements (Wang *et al.* 1997; Campbell & Waser 2001). However, the study of seedling success in these same hybrid systems reveals that environment-dependent selection acts differently against hybrid and parental species (Wang *et al.* 1997; Campbell & Waser 2001). Thus, the result that iris seeds and seedlings appear to be equivalent to those of their parent species is unique.

Seeds that had not germinated at the end of the experiment were almost universally still viable, suggesting that seeds are able to remain dormant until conditions are favourable for germination. We were caught off guard by the ability of seeds to remain viable while submerged. Iris seedlings are relatively rare in the wild, and nothing is known about the potential for seed banks to occur. There is no way of knowing from this experiment how long seeds can remain dormant in the wild. We now know that seeds transplanted into natural, sometimes submerged, conditions are capable of germinating 2 years after planting (Johnston 2002). Duration of seed dormancy will have to be addressed with a separate experiment in the future.

Because irises are clonal, they are able to adjust their position within habitat patches after they have established (Burke *et al.* 2000). Rhizomes are 10–20 cm in length (Viosca 1935), allowing a plant to 'walk' up or down slope, perhaps cueing in on favourable environmental conditions (Bazzaz 1991) and resulting in ecological zonation of adult irises (Cruzan & Arnold 1993; Johnston *et al.* 2001b). Similarly, Grace & 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. It seems likely that these irises are ecologically similar in the beginning, and selection at later stages drives the observed differences in habitat distribution among adult plants (Johnston *et al.* 2001b).

Previous studies have suggested that *I. brevicaulis* and *I. fulva* have different strategies of clonal and sexual reproductive allocation, and seed and seedling data support this idea. *Iris brevicaulis* exhibits a stronger

clonal strategy, making more and larger rhizomes and few and weaker seeds and seedlings. Due to differences in pollen receptivity, *I. brevicaulis* has been shown to be more likely to be the maternal parent of a hybrid cross (Cruzan & Arnold 1994). If hybrids were more likely to introgress towards *I. brevicaulis*, they may suffer from reduced fitness due to inherent weakness at the seedling stage of this species. However, many F₂B seed and seedling traits suggest that hybrids are vigorous at early stages in spite of *I. brevicaulis* as their cytoplasm parent. 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, F₁ 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 at multiple life stages in the field.

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