

ENVIRONMENT-DEPENDENT PERFORMANCE AND FITNESS OF *IRIS BREVICAULIS*, *I. FULVA* (IRIDACEAE), AND HYBRIDS¹

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We tested the relative fitness of two Louisiana *Iris* species (*Iris brevicaulis* and *I. fulva*) and their first-generation backcross hybrids in three experimental watering treatments: dry, field capacity, and flooded. Leaf area expansion rate, gas exchange (A_{\max} , g_s , c_i), and biomass at final harvest were measured for each species and hybrid class in all three environmental treatments. Fitness (based on total biomass) of the four genotypic classes differed significantly with environment. All genotypic classes performed most poorly in the dry treatment. The fitness ranking of genotypic class also changed across environments (significant genotypic class by treatment interaction) with hybrid genotype fitness shifting relative to parental genotypes. Integrating over all treatments, backcrosses to *I. fulva* showed the lowest fitness, whereas backcrosses to *I. brevicaulis* outperformed *I. fulva*. The differences in fitness were apparently achieved by a combination of differences in photosynthesis and allocation. In this system, hybrids are not necessarily less fit than their parents, and the relationship between hybrid and parental fitness is influenced by environmental conditions, lending support to the Hybrid Novelty model of hybrid zone evolution.

Key words: environment-dependent selection; hybrid fitness; hybrid novelty; hybrid zone; Louisiana *Iris*; photosynthesis.

In a hybrid zone, the reshuffling of gene combinations generates individuals with a range of genotypes and fitness levels (Anderson, 1949; Stebbins, 1959; Rieseberg et al., 1996; Arnold, 1997). To describe the resulting evolutionary patterns and trajectories, several models of hybrid evolution have been proposed (see Arnold, 1997, for a review). Four major models, the Tension Zone, Bounded Hybrid Superiority, Mosaic, and Hybrid Novelty, create a conceptual framework that generates hypotheses regarding mechanisms of hybrid fitness. Directly evaluating the goodness of fit between a given hybrid zone and a specific model of hybrid zone evolution can be problematic since several models predict similar spatial patterns of genotypic distribution. Testing the assumptions of hybrid zone models is more likely to lead to an understanding of the mechanisms behind the pattern (Moore and Price, 1993) and, thus, the model that best describes hybrid zone evolution in a particular case. While each model of hybrid zone evolution focuses upon a slightly different set of factors that influence fitness, there are two concepts that are covered by all: fitness of hybrid individuals relative to parental genotypes and the role of the environment as an influence on hybrid fitness (Hewitt, 1988; Arnold, 1997).

A defining characteristic of all hybrid zone models is the predicted potential fitness of hybrid genotypes relative to parental genotypes. In some models, hybrids may always be less fit than their parents due to genetic discontinuities (*Tension Zone*—Barton and Hewitt, 1985) or a lack of “hybrid” habitat

(*Mosaic*—Harrison, 1986; Howard, 1986). Other situations allow hybrids to be most fit relative to their parental species, but only in marginal or ecotonal habitat (*Bounded Hybrid Superiority*—Moore, 1977). In the final case, genetic recombination produces hybrids with variable fitness, including superior fitness to parental genotypes in certain environmental conditions (*Hybrid Novelty*—Arnold, 1997).

Hybrid zone models can be grouped into two categories based on their treatment of the environment as selection factor and, thus, hybrid zone evolution. Models that predict spatial patterns and relative fitness of hybrid genotypes within a hybrid zone using genetic interactions alone are based on environment-independent selection (Barton and Hewitt, 1985). Hybrids in environment-independent models are predicted to suffer from universally low fitness due to within-genome incompatibilities (Barton and Hewitt, 1985). Those that invoke genotype-by-environment interactions allow for environment-dependent selection (Moore, 1977; Harrison, 1986; Howard, 1986; Arnold, 1997). In an environment-dependent model, new genotypes created by recombination within a hybrid zone will be filtered by selection factors that vary with their surroundings (Rieseberg et al., 1996; Hatfield and Schluter, 1999; Hochwender and Fritz, 1999). If variation in environmental conditions exists within a zone, environment-dependent models would predict that fine-scale differences in selection pressure (e.g., Hamrick and Holden, 1979) should lead to variable relative fitness of a given genotype within the range of possible habitats in the hybrid zone (Harrison, 1986). The models that assume environment-independent selection would predict that environmental variation would not change the relative fitness of hybrids (Barton and Hewitt, 1985).

The first step in understanding the mechanisms underlying hybrid zone evolution is to examine the role of specific environmental factors in determining the fitness potential of hybrids relative to their parents (Arnold, 1997). The most straightforward approach is to isolate and manipulate environ-

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mental variables separately, measuring the relationship of each to selection observed in nature (Snow and Vince, 1984). New combinations of traits, loss of adaptive traits, and breaking up of trait complexes should influence ecological distributions of hybrids along environmental gradients (Endler, 1977; Arnold, 1997). In this regard, genetic recombination may potentially produce individuals with phenotypes that are intermediate to their parents (Riley, 1938) or transgressive and unique (Grant and Grant, 1996). An ideal system for studying the effects of the environment on hybrid fitness should therefore possess identifiable environmental gradients within the hybrid zone.

Hybridization within the Louisiana *Iris* species complex (series Hexagonae) has been studied for over half of a century (Viosca, 1935; Anderson, 1949; Bennett, 1989; Arnold, 1992). The distributions of all Louisiana *Iris* species overlap in the bayous of southern Louisiana, where previous descriptions of the species complex indicate ecological differentiation (Viosca, 1935; Riley, 1938). As in any wetland system, depth and duration of flooding play a substantial role in structuring the bayou and swamp communities in which the Louisiana irises live. Such a natural gradient presents the opportunity to study plant adaptation to flooding (reviewed in Blom et al., 1994; Blom and Voesenek, 1996; Crawford, 1996) and resulting zonation of vegetation (Hoagland and Collins, 1997; Kotowski, van Diggelen, and Kleinke, 1998; Howard and Mendelsohn, 1999).

We have chosen *Iris brevicaulis*, *I. fulva*, and *I. brevicaulis* × *I. fulva* hybrids for the present investigation of hybridization and its potential ecological ramifications. Much is already known about the hybridization between *I. fulva* and *I. brevicaulis*. First, a multivariate analysis of abiotic and biotic field data revealed habitat associations for *I. fulva*, *I. brevicaulis*, and their hybrids in natural settings (Cruzan and Arnold, 1993). Second, a greenhouse study revealed that, under optimal conditions, various hybrid genotypes exhibited a spectrum of fitness (Burke, Voss, and Arnold, 1998). Finally, reciprocal transplants have been made for the two parental species and several types of hybrid progeny to investigate fitness differences across their range of natural habitats (Wesselingh, Johnston, and Arnold, unpublished data). Thus, while it has been shown that the species of Louisiana *Iris* tend to live in different habitats (Cruzan and Arnold, 1993), it is yet to be demonstrated that they are most fit in their "own" environment. In addition, no single environmental cline has been identified that explains genotypic assortment across habitats.

For another type of hybrid cross (*Iris hexagona* × *I. fulva*) field surveys (Emms and Arnold, 1997), reciprocal transplants (Emms and Arnold, 1997), and molecular analyses (Burke, Carney, and Arnold, 1998) show that both environment-dependent and -independent selection are acting on hybrid genotypes. These types of selection affect the distribution of the two species and their hybrids within the habitats available to them (Bennett and Grace, 1990; Arnold and Bennett, 1993; Emms and Arnold, 1997; Burke, Carney, and Arnold, 1998).

In this study, we begin the investigation of individual environmental gradient effects on the fitness of *I. fulva*, *I. brevicaulis*, and hybrid progeny under experimental conditions. We estimated fitness from total biomass and measured growth and gas exchange of *I. fulva*, *I. brevicaulis*, and first-generation backcrossed progeny across three watering treatments. The present study thus addresses the following questions about these four genotypic classes: (1) is estimated hybrid fitness always lower than that of parental species, and (2) does water

level affect fitness, growth rate, and gas exchange of hybrids relative to parent species in this Louisiana *Iris* hybrid cross?

MATERIALS AND METHODS

Preparation of plant material—Four genotypic classes were used for this study, two species (*I. brevicaulis*, *I. fulva*) and two types of hybrids (reciprocal first-generation backcrosses between *I. brevicaulis* and *I. fulva*). Hereafter genotypic classes will be referred to as IB, IF, BCIB, and BCIF, respectively. The IB and IF plants were propagated from collections originally taken from the field as rhizomes (IB—St. Martin Parish, Louisiana, USA; IF—Terrebonne Parish, Louisiana, USA) and maintained at the University of Georgia Botany greenhouse. The BCIB and BCIF individuals were the result of experimental crosses performed in the greenhouse in 1996. During the course of each growing season, old rhizome material is spent and new tissue is formed. Each fall, individuals from all four genotypic classes of *Iris* were re-propagated using from rhizome material formed during the previous growing season. In this way, the carryover effects from experiencing different environments prior to collection were minimized.

On 8 April 1998, rhizomes of IB, BCIB, BCIF, and IF were excised from mature greenhouse-grown plants and cut into sections ~5 cm long. Roots and leaves were trimmed to within 1 cm of the rhizome. Each rhizome piece was rinsed and then blotted dry. To account for variation in rhizome size within and among genotypic classes, rhizome mass was measured before planting. Genotypic class differences in initial rhizome mass were analyzed using one-way ANOVA procedures (PROC GLM, SAS, 1989). Rhizome pieces were individually planted into 18-cm diameter pots filled with a standard pine bark soil. (Each batch of pine bark soil consisted of: 23.7 L [25 quarts] fine-grade composted pine bark, 7.6 L [8 quarts] coarse-grade vermiculite, 118.3 cm³ [8 TBS] powdered lime, 29.6 cm³ [2 TBS] superphosphate, 14.8 cm³ [1 TBS] calcium nitrate, 14.8 cm³ [1 TBS] potassium nitrate, 14.8 cm³ [1 TBS] gypsum, 14.8 cm³ [1 TBS] "Micromax" micronutrient.) A cap of 200 cm² of sand was added to each pot to keep the pine bark mix in place during watering. Plants were maintained in the greenhouse for 8 wk with daily watering before differential watering treatments were initiated. Plants that survived transplantation were randomly assigned to one of three watering treatments.

At watering treatment initiation on 2 June, number of leaves, leaf area, and number of growing points (shoot meristems producing at least one leaf >5 cm in length) were measured. Leaf area was estimated from leaf length and width at a point approximately halfway up the leaf. The relationship between estimated leaf area and measured leaf area (LI-3100 Area Meter, LI-COR, Lincoln, Nebraska, USA) was confirmed at the end of the experiment. Genotypic class differences in shoot characteristics at treatment initiation were analyzed by ANOVA and ANCOVA procedures (PROC GLM), using initial rhizome biomass in the ANCOVA. Treatment means were compared in both cases with Tukey's test statistics.

Watering treatment experiment—Plants were exposed to three watering treatments (dry, field capacity, and flooded) from 2 June to 30 July 1998. Dry treatment plants were watered every 4th d, field capacity plants were watered daily and allowed to drain, and flooded plants were submerged in standing water to the soil surface. Leaf area was measured eight times at weekly intervals following water treatment initiation. Increase in leaf area over time was used to calculate a nondestructive estimate of relative growth rate (RGR).

Leaf gas exchange characteristics (instantaneous photosynthetic rate [A_{max}], leaf conductance [g_s], and intercellular CO₂ concentration [c_i]) were measured for all plants on 28–30 July 1998 (LI-6400 Portable Photosynthesis System, LI-COR, Lincoln, Nebraska, USA). Standard conditions for measurements were 350 μmol/mol CO₂, saturating light level 2000 μmol · m⁻² · sec⁻¹, and leaf temperature 30°C ± 0.3°C. Measurements were taken once leaf parameters had stabilized (<1% total system coefficient of variation, change of CO₂ and H₂O signals over time) after 3–5 min. Gas exchange measurements were made on three consecutive sunny mornings. Plants in the dry or field capacity treatments were measured on a day they were watered.

At the end of the experiment, plants were harvested to assess biomass. Shoots were severed at the soil surface and roots and rhizomes recovered by

TABLE 1. Leaf area results (from SAS PROC MIXED) with leaf area at treatment initiation as the covariate. Numerator degrees of freedom and denominator degrees of freedom are given for each statistical effect. A significant Genotypic class \times Time interaction would indicate a genotypic class difference in nondestructive relative growth rate. *P* values < 0.05 are in boldface type.

Source	df	<i>F</i>	<i>P</i>
Covariate (Leaf area)	1, 763	1670.62	0.001
Water level	2, 4	33.19	0.003
Genotypic class	3, 763	7.95	0.001
Water level \times Genotypic class	6, 763	13.69	0.001
Time	7, 763	118.31	0.001
Water level \times Time	14, 763	7.62	0.001
Genotypic class \times Time	21, 763	0.79	0.717
Water level \times Genotypic class \times Time	42, 763	1.26	0.142

gently washing away the pine bark mix and removing the remaining debris by hand. Roots, rhizomes, and leaves were dried at 60°C and then weighed.

To test for aerenchyma formation, tissue density was measured in a subsample of roots and rhizome from a subset of randomly selected plants. Samples of ~1 g root and ~10 g rhizome were inserted into a water-filled graduated cylinder modified to function as a Eureka apparatus (Curran, James, and Allaway, 1996). Tissue volume was determined, samples were blotted dry, fresh mass measured, and density calculated (density = grams wet mass/cm³ water displaced). Tissue subsamples were then returned to the sample from which they came.

Statistical analyses—The experiment was a split-plot design, with Water level as the whole-plot factor, and plant Genotypic class (IB, BCIB, BCIF, and IF) as the subplot factor. Block and block interactions were random factors, necessitating a mixed model: SAS, PROC MIXED (SAS, 1989; Littell et al., 1996).

Gas exchange and root density data were analyzed with ANOVA procedures (PROC MIXED) using Genotypic class and Water level as main effects. Because gas exchange and root density are measured per unit area and per unit volume, respectively, there was no need to construct the statistical model to account for initial size differences among classes.

Leaf area expansion and final biomass were analyzed with leaf area at treatment initiation as a covariate. For these characters, photosynthetic surface at treatment initiation seemed the most appropriate estimate of starting potential (Lambers, Chapin, and Pons, 1998). Leaf area expansion was analyzed with repeated-measures ANCOVA in SAS, (PROC MIXED) using Water level, Genotypic class, and Time as main effects.

Total biomass and biomass partitioning were analyzed with ANCOVA (PROC MIXED) with Water level and Genotypic class as main effects. A significant Genotypic class \times Water level interaction precluded statistical comparison of individual biomass means among treatment combinations, but differences were interpreted visually as an indicator of relative performance and fitness.

RESULTS

Starting conditions—Genotypic classes differed significantly in initial rhizome fresh mass. *Iris brevicaulis* initial rhizome biomass (mean \pm 1 SE, 27.50 \pm 10.07 g) was significantly greater than that of the other genotypic classes (20.43 \pm 9.58 g, 17.58 \pm 7.07 g, 20.72 \pm 8.96 g, for BCIB, BCIF, and IF, respectively [df = 3, *F* = 5.53, and *P* = 0.014]).

Shoot characteristics were measured at watering treatment initiation (2 June). These included number of growing points, number of leaves, and leaf area. For each of these variables, the covariate of initial rhizome mass accounted for a significant amount of variation (*P* \leq 0.001 for all three characters). There were no significant differences (*P* < 0.05) among genotypic classes in the number of growing points (1.34–1.59) or number of leaves per plant (4.7–5.7). However, the genotypic classes did differ significantly for leaf area at this time

(df = 3, *F* = 4.44, *P* = 0.0054). At the initiation of the watering treatments, the leaf area was greatest for BCIF (158.68^a \pm 14.86) and progressively lower for BCIB, IF, and IB (110.59^{ab} \pm 15.56, 96.92^b \pm 14.44, and 82.09^b \pm 18.91, respectively), a reversal of genotypic class ranking compared to initial rhizome mass. Variation present at treatment initiation was accounted for statistically by using leaf area at treatment initiation as a covariate in analyses of the experimental treatment effects.

Water treatment effects—Leaf area at treatment initiation (covariate) was a significant factor explaining variation in relative growth rate (RGR), as were Water level, Genotypic class, Time, and several interaction terms (Table 1). However, the lack of significance for the Genotypic class \times Time interaction term indicates that the genotypic classes did not differ significantly in rate of relative leaf area expansion from the point of treatment initiation. Thus, the average leaf area during the water treatment, adjusted for leaf area at treatment initiation, is presented (Fig. 1B). Although there are no significant genotypic class differences in this nondestructive relative growth rate, the patterns in the data mirror trends in biomass accumulation.

The gas exchange parameters A_{\max} , g_s , and c_i showed little difference among Genotypic class and Water level treatments, except for a significant effect of genotypic class on A_{\max} (Table 2). The BCIF class had a lower A_{\max} than any other genotypic class (Table 3). Across Genotypic class and Water treatments, g_s and c_i were not statistically different. Only the Genotypic class effect was significant for root density (Table 2). Both IB and IF had higher root density than the hybrid classes of BCIB and BCIF (Table 3).

At final harvest, the effects of Leaf area (covariate), Water level, and Water level \times Genotypic class were significant for total dry biomass and its components of leaf, rhizome, and root biomass (Table 2). There were also significant differences among genotypic classes for total and rhizome biomass. When adjusted total biomass is plotted separately by Genotypic class and Water level, it is clear that biomass is lower in the dry treatment as compared to the other treatments, and that the increased growth of IB in the flooded treatment contributes to the significant Water level \times Genotypic class interaction (Fig. 1A). Biomass ratios of root/total (0.05–0.09) and belowground (root + rhizome)/total (0.66–0.76) did not differ significantly for Genotypic class or Water level treatment.

DISCUSSION

In the present study, fitness estimated from total biomass was shown to be variable for first-generation backcross hy-

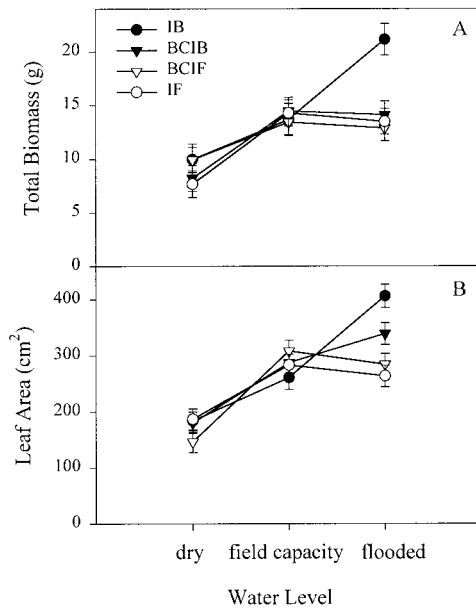


Fig. 1. (A) Total biomass (mean \pm 1 SE) at final harvest for dry, field capacity, and flooded watering treatments. Means have been adjusted for leaf area at treatment initiation. For genotypic class, IB is *Iris brevicaulis*, IF is *Iris fulva*, and BCIB and BCIF are hybrids (first generation backcrosses of an F₁ hybrid to IB and IF, respectively). For statistical results see Table 2. (B) Mean leaf area (mean \pm 1 SE) of IB, BCIB, BCIF, and IF over 8 wk of growth in three watering treatments. Means are adjusted for leaf area at treatment initiation. For statistical results see Table 1.

brids of IB and IF. Fitness ranking of hybrid classes varied from highest to lowest, depending on the watering treatment. Previous observations and habitat descriptions led to the prediction that IF was more flood tolerant than IB (Viosca, 1935; Arnold, 1994). Our results largely disagreed with predicted habitat preferences and corresponding physiological tolerances. While soil water condition did seem to influence relative performance and fitness of Louisiana irises, it alone could not explain distribution patterns of IB, IF, and their hybrids in natural settings.

Some genotypic class differences were apparent prior to watering treatment initiation. *Iris brevicaulis* had significantly larger initial rhizome mass than IF, BCIF, and BCIB, which

were statistically indistinguishable from each other. Differences in rhizome length may have influenced the number of nodes, number of potential growing points, and subsequent growth patterns independent of biomass. In addition to biomass, inherent differences in rhizomes among genotypic classes might have affected the outcome. Genotypic class differences in early-stage developmental timing, similar to those demonstrated for peak flowering time (Arnold, 1994) might explain the reversal of genotypic class ranking for initial rhizome biomass and leaf area at treatment initiation.

The quality of resources stored in rhizomes may vary between genotypic classes causing differences in growth and biomass patterns (Hanhijarvi and Fagerstedt, 1995). Rhizome differences were unlikely due to variation in environments previously experienced by each genotypic class. While IB and IF were collected in the field, and BCIF and BCIB were created in the greenhouse, the annual propagation of each individual from rhizome tissue that was formed within the common greenhouse environment should have minimized the carryover of environmentally induced differences.

At treatment initiation, genotypic classes differed significantly in leaf area. Because leaf area at treatment initiation probably reflected many factors, including rhizome size, environmental condition following transplantation, and developmental timing of genotypic classes, it seemed biologically to be the most appropriate covariate in later analyses. Rigorous tests of statistical assumptions (reviewed in Huitema, 1980) also indicated leaf area at treatment initiation is the best covariate. Using leaf area at treatment initiation as a covariate effectively equalized all genotypic classes at that point. This allows for the most conservative assessment of differences that occurred only during the duration of watering treatments.

Fitness effects—Total biomass was chosen to estimate fitness because it is known to correlate well with contribution of offspring to the next generation (Bazzaz et al., 1987) making it the best single-season indicator of fitness for a perennial, clonal, herbaceous plant (Wikberg, Svensson, and Carlsson, 1994; Cheplick, 1995). Biomass measures used in our analyses were adjusted for leaf area at treatment initiation (Table 2) to best reflect the changes that occurred due to growth during the treatment period. Fitness estimates did vary among genotypic classes. Hybrids were similarly fit to IF on average (Table 4).

TABLE 2. ANOVA and ANCOVA results (*F* and *P* from SAS PROC MIXED) for final harvest biomass, root density, and gas exchange parameters at the midpoint of treatments (*A*_{max} is photosynthesis, *g*_s is stomatal conductance, and *c*_i is intercellular CO₂). For biomass characters, df (numerator/denominator) are 1/103, 2/4, 3/103, and 6/103 for the effects of Leaf area at treatment initiation (covariate), Water level, Genotypic class, and Water level \times Genotypic class, respectively. For *A*_{max}, *g*_s, and *c*_i, the df (numerator/denominator) are 2/4, 3/102, and 6/102 for Water level, Genotypic class, and Water level \times Genotypic class, respectively. For root density the df are 2/1, 3/11, and 6/11 for Water level, Genotypic class, and Water level \times Genotypic class, respectively. *P* values < 0.05 are in boldface type.

Source	Covariate		Water level		Genotypic class		Water level \times Genotypic class	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Total biomass	343.52	0.001	28.68	0.042	3.70	0.014	3.28	0.005
Leaf biomass	185.53	0.001	27.16	0.005	0.34	0.794	2.35	0.036
Rhizome biomass	292.47	0.001	19.22	0.009	6.46	0.001	2.79	0.015
Root biomass	166.99	0.001	15.26	0.013	0.22	0.883	2.71	0.017
Belowground/Total biomass	0.85	0.357	5.10	0.079	1.69	0.174	0.89	0.503
Root/Total biomass	2.37	0.127	0.78	0.518	2.29	0.082	0.61	0.720
<i>A</i> _{max} ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	—	—	3.53	0.121	2.83	0.042	0.67	0.671
<i>g</i> _s ($\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	—	—	1.75	0.285	1.42	0.240	1.42	0.215
<i>c</i> _i (ppm)	—	—	2.48	0.199	1.29	0.282	1.06	0.390
Root density (g/cm^3)	—	—	5.78	0.282	11.29	0.001	1.15	0.396

TABLE 3. Genotypic class values (mean \pm 1 SE) for gas exchange characters and root density. Different superscripts indicate significant differences. $N = 22, 34, 34,$ and 38 for genotypic classes IB, BCIB, BCIF, and IF (see *Materials and Methods: Preparation of plant material* for explanation of abbreviations), respectively, for gas exchange characters. For root density, $N = 6-7$ for each genotypic class.

	IB	BCIB	BCIF	IF
A_{\max} ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	15.6 ^a \pm 0.6	14.1 ^a \pm 0.5	13.5 ^b \pm 0.5	14.7 ^a \pm 0.5
g_s ($\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	0.34 ^a \pm 0.02	0.33 ^a \pm 0.02	0.32 ^a \pm 0.02	0.37 ^a \pm 0.02
c_i (ppm)	244 ^a \pm 18	217 ^a \pm 15	244 ^a \pm 14	256 ^a \pm 14
Root density (g/cm^3)	0.83 ^a \pm 0.02	0.73 ^b \pm 0.02	0.75 ^b \pm 0.02	0.85 ^a \pm 0.02

In contrast, IB attained a much higher biomass than all other genotypic classes.

Growth and biomass differences can often be traced to the individual traits that contribute to these factors. In this study, photosynthetic rate (A_{\max}) was the only ecophysiological character that showed significant variation among genotypic classes. The statistical difference was driven by a significantly lower A_{\max} for BCIF than the other three genotypic classes. Reduced A_{\max} likely contributed to the lowest average fitness ranking for BCIF (Table 4). However, A_{\max} in BCIB was similar to IB and IF, suggesting that reduced photosynthesis is not the general case for all hybrid individuals or classes (Table 3). Nondestructive relative growth rate (estimated from leaf area expansion) did not differ among genotypic classes (Table 1), although an examination of the data indicated a trend that matched biomass accumulation. Taken as a whole, these individual characters suggest that differences in biomass were achieved through a combination of differences in photosynthesis and allocation.

Environment effects—Significant interactions of watering treatment and genotypic class on final biomass suggest that performance of both hybrid and parental species individuals is dependent on environmental conditions. Estimated relative fitness was highest for IB in the flooded treatment, and all other genotypic classes attained maximum biomass in the field capacity treatment. Among watering environments, all genotypic classes show lowest fitness in the dry treatment. In the field capacity treatment, both hybrids had fitness rankings higher than parental species (Fig. 1A, Table 4). However, the two hybrid classes experienced a greater reduction in performance in the dry treatment than the two parental species, producing a lower relative fitness ranking.

The reversal of the relationship of hybrid and parental species (in terms of biomass) across the three watering treatments indicates that there is an effect of the environment on the fitness of Louisiana *Iris* species and their hybrids. Average leaf area in each treatment mirrored biomass patterns, demonstrating that the two were closely related (Fig. 1B). While leaf area differences were not statistically significant due to high levels

TABLE 4. Relative fitness ranking for genotypic classes of Louisiana *Iris* species and hybrids based on total biomass achieved after 8 wk of being subjected to dry, field capacity, and flooded conditions.

Genotypic class	Dry	Field capacity	Flood	Average
<i>Iris brevicaulis</i>	1	3	1	1
BCIB ^a	3	1	2	2
BCIF ^b	4	2	3	4
<i>Iris fulva</i>	2	4	4	3

^a BCIB = first generation hybrid backcrosses toward *I. brevicaulis*.

^b BCIF = first generation hybrid backcrosses toward *I. fulva*.

of within-genotypic class variation, the similarity between leaf area and biomass reinforces the observed fitness pattern.

The flooding treatment in the experiment was expected to be the most stressful watering condition. Flooding restricts gas movement through the soil, starves roots for oxygen, and alters soil properties (Armstrong, Brändle, and Jackson, 1994; Drew, 1997). There are various adaptations to flooding, each individual plant potentially employing multiple strategies (Armstrong, Brändle, and Jackson, 1994; Blom and Voisenek, 1996; Crawford, 1996; Drew, 1997). Comparisons of closely related species occurring along water gradients have demonstrated that small changes in adaptive strategy are very important to ecological distribution and can lead to habitat differentiation within genera (*Typha*—Grace and Wetzel, 1982; *Rumex*—Visser, Blom, and Voisenek, 1996; *Carex*—Moog, 1998; *Ranunculus*—He et al., 1999). More air space in roots has been shown to allow metabolism to continue in the face of anoxia in some flood tolerant plants (Armstrong, Brändle, and Jackson, 1994; Drew, 1997). Root density was lowest in hybrids, indicating they should have been better equipped to aerate their roots in flooded conditions. Differences in root density in these Louisiana irises did not correlate with increased performance in the flooded treatment, so perhaps the slight variation in root density was not biologically relevant.

Conclusions—Our data demonstrated that both genetic make-up and watering treatment had an effect on growth and fitness of Louisiana irises. In his description of the hybrid novelty theory, Arnold (1997) followed the lead of Anderson and Stebbins (1954) suggesting that hybrid genotypes can possess a wide range of fitness relative to parental species. Thus, relative fitness of individuals is predicted to change across environmental conditions due to novel traits or combinations of traits that can arise during hybridization. Investigations of fitness variation due to genetic incompatibility in Louisiana irises (Burke, Voss, and Arnold, 1998) set the stage for our ecological study that took into account genetic and environmental factors.

The findings of the present study lend indirect support to the applicability of the hybrid novelty theory (Arnold, 1997) for the Louisiana irises. Ecophysiological traits (leaf area expansion, gas exchange, root density) in hybrids were shown to be both intermediate and transgressive. There did not appear to be a relationship among responses of traits within a genotypic class. Fitness, as estimated by final biomass, was not consistently lower in hybrid classes. Both relative fitness of hybrids, as well as other traits, varied across three different soil moisture treatments. Furthermore, genotype by environment interactions produced variable relative fitness and performance of genotypic classes that depends on their environmental setting. These results indicate that the potential exists

for the production of hybrid genotypes that are better suited to some habitat types than the parental species.

While soil moisture condition does not definitively explain the distributions of IB, IF, and their hybrids, it does suggest that the environment exerts a selective and therefore organizing force on these plants. Ongoing analyses of additional abiotic factors (e.g., shade and soil properties), biotic factors (e.g., competition and herbivory), and interactions among factors should inform our understanding of adaptive ecophysiological characteristics in Louisiana irises and how they drive zonation patterns seen in nature.

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