Journal of Ecology 2004
92, 967–976

Novel phenotypes among early generation hybrids of two Louisiana iris species: flooding experiments

JILL A. JOHNSTON*, LISA A. DONOVAN and MICHAEL L. ARNOLD†
Department of Plant Biology and †Department of Genetics, University of Georgia, Athens, GA 30602, USA

Summary

1 Recent studies of hybridization have shown that extreme (e.g. transgressive) phenotypes can be generated during massive genetic recombination events such as interspecific hybridization. Extreme hybrid phenotypes appear to have the potential to create new lineages and lead to evolutionary divergence.

2 Hybrid genotypes of some salt marsh species have proven capable of reengineering their ecosystems, while in other cases, hybridization has transformed plant communities by facilitating species invasion.

3 Using the Louisiana irises, a naturally hybridizing group of species, we assessed the potential for hybrids of *Iris brevicaulis* and *I. fulva* to possess extreme physiological and growth traits. Our glasshouse experiment used two soil water environments (wet and flood) to measure hybrid trait expression at both the genotypic class and individual genotype level.

4 At the genotypic class level, two of the three hybrid classes (F_1s, and backcrosses towards each parental species) were transgressive for at least one physiological trait (F_1s for root mass proportion, specific leaf area and final leaf area; backcrosses to *I. brevicaulis* for leaf area ratio). Three fitness components (total biomass, ramet production and flower production) were measured on each plant in the experiment. All three hybrid classes had greater clonal fitness (total biomass) but not greater sexual fitness (flower number) than parent species.

5 At the individual genotype level, two F_1 hybrids showed extreme physiological trait expression: one for specific leaf area, and the other for shallow root allocation. Flower production was the only fitness component that showed some degree of environment-dependence at the individual genotype level.

6 Although early generation hybrid classes can contain rare genotypes with extreme phenotypic trait expression, and hybrid fitness components were equal or superior to both parental species in most cases, there was not a strong association between transgressive traits and elevated hybrid fitness.

Key-words: clonal fitness, flood tolerance, hybrid fitness, hybrid novelty, hybrid zone, hybridization, Louisiana iris, phenotypic plasticity, transgressive traits, wetland

Journal of Ecology (2004) **92**, 967–976

Introduction

The role of interspecific hybridization as a creative force in evolution is receiving renewed attention from evolutionary biologists. Contrary to the opinion that was common among architects of the modern synthesis (Dobzhansky 1940; Mayr 1942; Barton & Hewitt 1985), recent studies suggest that successful hybrids are relatively common in nature (Rieseberg & Ellstrand 1993; Rieseberg 1997; Arnold *et al*. 2001) and can possess novel phenotypic traits and allele combinations that are not present in either parent species (Anderson & Stebbins 1954; Lewontin & Birch 1966; Arnold 1992; Rieseberg 1997; Rieseberg *et al*. 1999; Burke & Arnold 2001; Schwarzbach *et al*. 2001). The traditional expectation that hybrids will be phenotypically intermediate has been supported by many studies (Riley 1938; Hatfield & Schluter 1999; Campbell & Waser 2001). However, several recent examples of extreme phenotypic traits among hybrid individuals and hybrid species

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*Present address and correspondence: Jill A. Johnston, Department of Plant Biology, University of Minnesota, St Paul, MN 55108, USA (e-mail john6388@umn.edu).

have been found in plants (Arnold 1992; Ellstrand & Schierenbeck 2000; Schwarzbach *et al*. 2001; Rosenthal *et al*. 2002; Rieseberg *et al*. 2003).

The evidence from natural populations suggests that rare, successful hybrids with extreme phenotypes may play a significant role in plant evolution by causing the ecological expansion of one parental species following introgression (Lewontin & Birch 1966), ecological divergence of the hybrid lineage (Grant & Grant 1996) and speciation (Arnold 1993; Rieseberg *et al*. 2003). Additionally, several recent studies show hybridization facilitating species invasion (Ellstrand & Schierenbeck 2000) and creating phenotypes with unique enough ecologies to reengineer the ecosystem in which they occur (Daehler & Strong 1997; Anttila *et al*. 2000; Figueroa *et al*. 2003). For extreme phenotypic traits to persist in a hybrid lineage, they must have a positive or neutral effect on relative hybrid fitness (Arnold 1997). Physiological traits that govern a plant's ability to grow and capture resources are likely to be closely linked to performance and fitness (Larcher 1995; Lambers *et al*. 1998). Extreme physiological traits are likely to result in hybrid individuals with ecological relationships that differ from both parent species (Arnold 1997; Arnold *et al*. 2001; Schwarzbach *et al*. 2001; Figueroa *et al*. 2003; Rieseberg *et al*. 2003). Hybrid novelty of physiological traits will be subject to environmental selection, potentially allowing some hybrid individuals to have high environment-dependent fitness relative to parent species (Arnold 1997).

Hybridization has already left its mark on the ecology of the Louisiana iris species complex (Arnold 2000), a group of rhizomatous perennial plants whose distributions overlap in southern Louisiana (Viosca 1935; Arnold 1994). Two of the species, *Iris brevicaulis* Walker and *Iris fulva* Ker-Gawler, are distinct floristically, yet similar enough ecologically to co-occur and hybridize (Cruzan & Arnold 1993; Johnston *et al*. 2001b). Both species occur along the edges of bayous and swamps, but in different microhabitats (Cruzan & Arnold 1993). *Iris brevicaulis* grows in areas that have more sunlight, while *I. fulva* thrives in shaded, wetter habitats (Johnston *et al*. 2001a).

These *Iris* species can colonize diverse microhabitat patches by seed or vegetative rhizomes (Cruzan & Arnold 1993; Burke *et al*. 2000; Johnston *et al*. 2001b). Sexual reproductive fitness of hybrids between *I. brevicaulis* and *I. fulva* varies among genotypes, but field and glasshouse studies suggest that average hybrid fitness is fairly equal to that of parent species (Burke *et al*. 1998; Wesselingh & Arnold 2000). Although Louisiana iris seedlings appear to be rare in the field (Johnston, Wesselingh & Arnold, unpublished data), genetic diversity in populations is high (Burke *et al*. 2000). Sexual reproduction appears to be important in some years, but traits contributing to survival and clonal fitness are also important to the lifetime fitness of Louisiana iris genotypes.

In this study, we have measured physiological traits and fitness components of *I. brevicaulis*, *I. fulva* and their early generation hybrids to assess hybrid traits and fitness. Specifically, we asked three questions. First, is there transgressive expression of physiological and growth traits in hybrids between *I. brevicaulis* and *I. fulva*? Secondly, does hybrid fitness relative to parental species change across environments? Thirdly, are transgressive or novel hybrid traits associated with fitness of hybrid genotypes? These three questions were explored at both the genotypic class level and the individual genotype level. We examine our findings at both levels, and ask whether there are consistent trends.

Materials and methods

PLANT MATERIAL

Two species, *Iris brevicaulis* (IB) and *I. fulva* (IF), and three genotypic classes of 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, USA). Rhizomes were transplanted into potting soil and grown under well-watered, fertilized conditions in the University of Georgia Botany Department glasshouse (Athens, GA, USA). In early spring 1995, crosses were initiated between IB and IF to produce F_1 hybrids. Seeds from these crosses were planted in autumn 1995. In spring 1996, pollen from F_1 flowers was crossed onto IB and IF stigmas to produce first generation backcrosses. First generation backcross hybrid seeds were planted in the glasshouse in autumn 1996. Hybrid genotypic classes will hereafter be referred to as F_1, BCIB (backcross towards *I. brevicaulis*) and BCIF (backcross towards *I. fulva*). All five genotypic classes were maintained in the glasshouse under similar conditions until September 1998.

Plant material was available for a limited number of genotypes in each genotypic class (IB, IF, F_1, BCIB and BCIB). To maximize the information gained from the experiment, we chose only five genotypes to represent each genotypic class. On 4–5 September 1998, rhizome material produced during the 1997–98 growing season was trimmed of leaves and roots, and divided into individual ramets. Initial fresh weight of rhizomes was recorded, and each rhizome segment was planted into an 18-cm diameter pot filled with pine bark potting mix. Pots were placed onto a glasshouse bench and allowed to establish in sunny, well-watered conditions for 6 weeks.

On 28 October 1998 six ramets (all with at least one leaf > 10 cm in length) were selected from each of the 25 genotypes for use in the experiment. Thus, there were 150 pots in the experiment. From each genotype, three ramets were assigned to each of two water treatments, wet and flood.

Rhizomes from spontaneous hybrids generated in a natural setting were not used in this experiment for two reasons. First, many hybrid generations are present in a natural hybrid swarm, and it would have been difficult to classify or replicate genotypic groups in a meaningful way. Secondly, field-collected rhizomes would have needed several years of growing and propagation in glasshouse conditions to ensure that they had lost any effects of canalization or exposure to their previous environment, and time did not permit such an acclimation.

WATER TREATMENTS

The three ramets of each genotype assigned to the wet treatment were placed into standing water level with the surface of the soil in the pots. Ramets assigned to the flood treatment were placed in standing water that was 10 cm above the soil surface. Large plastic tubs with holes drilled at the appropriate height were used to maintain treatment water levels. Tubs were kept full by top watering with a hose three times each week. Each tub held five pots, with one representative ramet from each genotypic class (IB, IF, F_1, BCIB, BCIF). To maintain a low dissolved oxygen concentration in the flood treatment, nitrogen gas (N_2) was bubbled through the water in the relevant tubs for 20 minutes following watering. Throughout the experiment, relative humidity, ambient light level, and air and water temperature, were monitored.

RESPONSE VARIABLES

Several characters were measured on each plant during the course of the experiment. Leaf area was measured at 5-week intervals as a non-destructive estimate of growth rate (28 October, 2 December, 6 January, 10 February, 17 March, 21 April). We estimated area of each leaf by measuring its length (to closest cm) and width at midpoint (to nearest mm) and calculating the area of the resulting rectangle. Leaves > 5 cm in length were included in the leaf area estimate for each plant. To verify the accuracy of the estimation procedure, leaf areas from a subset of plants were measured directly with the LI-3000 leaf area meter (Li-Cor, Lincoln, NE, USA) following final harvest (21 April). Regression analysis in Sigma Plot 2000 (SPSS Science, Chicago, IL, USA) indicates a strong correlation between leaf area estimates and actual leaf area ($r^2 = 0.89–0.92$).

On 21 and 22 April 1999, all plants were harvested. Leaves and flower stalks were cut at the rhizome surface, and roots and rhizomes washed free of soil. Leaves, rhizomes, roots and flower stalks were separated and dried at 60 °C to constant mass. Biomass allocation was evaluated from final dry weights: we determined leaf mass proportion (leaf mass/total biomass), rhizome mass proportion (rhizome mass/total biomass) and root mass proportion (root mass/total biomass). Leaf area ratio (LAR = leaf area/total biomass) and specific leaf area (SLA = leaf area/leaf biomass) were calculated from estimated leaf areas and biomass at harvest. Two types of flood-specific responses, shallow root ratio and tissue density of roots and rhizomes, were measured at harvest. Shallow root ratio is a proxy for shifts in root allocation into upper, more oxygenated soil layers. To measure shallow root ratio, 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 root biomass/total root biomass) was calculated. A small amount of fresh root and rhizome tissue was set-aside during harvest for tissue density measurements. Low tissue density indicates the presence of aerenchyma, air channels that supply submerged tissue with oxygen. All plants were subsampled for root tissue density and a subset of plants were subsampled for rhizome density. Tissue density was measured with a Eureka apparatus, using the water displacement method of Curran *et al.* (1996). Tissue subsamples were returned to their parent samples prior to drying.

Three fitness components were measured at the end of the experiment: total biomass, number of rhizomes produced and number of flower stalks. There are several fitness components that contribute to lifetime fitness of a clonal plant, which is ultimately reflected in the number of ramets per genet present in a population at any given time (Harper 1977; Wikberg 1995; Gardner & Mangel 1999; Winkler & Fischer 1999). We counted the number of ramets produced by each plant as an estimate of clonal fitness (Wikberg 1995). Total biomass was also measured following harvest to estimate each plant's ability to grow and acquire nutrients. Biomass is often used as an integrative measure of clonal fitness (Harper 1977; Solbrig 1981; Mendez & Obeso 1993; Wikberg *et al.* 1994). New flower stalks and flowers were recorded three times per week as an estimate of potential sexual reproduction (Wesselingh & Arnold 2003).

EXPERIMENTAL DESIGN

The experiment had a split-plot design with water treatment (two levels, wet and flood) as the whole plot factor and genotypic class (five levels, IB, IF, F_1, BCIB, BCIF) as the sub-plot factor. Pairs of plastic tubs (one wet and one flood treatment tub, with each tub containing one plant from each genotypic class) were arranged along a glasshouse bench. Each of the 15 pairs of tubs contained a replicate of the experimental treatment and units, and was treated as a block.

STATISTICAL ANALYSES

Genotypic classes

All statistical analyses were performed with SAS statistical software (SAS Institute, Cary, NC, USA) using PROC MIXED (SAS 1989). PROC MIXED was chosen over more traditional sums of squares procedures (PROC GLM) because it explicitly models random effects (Littell *et al.* 1996). For our analyses, block and block × water interactions were modelled as random.

For genotypic class level analyses, all ramets were treated as independent experimental units.

After analyses of main effects, trait expression among genotypic classes was compared. Overall means of each genotypic class were compared using independent contrasts to evaluate transgressive or extreme trait expression (Schwarzbach *et al*. 2001; Lexer *et al*. 2003). Hybrid genotypic classes were considered transgressive when trait expression of the hybrid class was extreme relative to IB and IF, and the hybrid class mean was significantly different from both parents. Physiological traits that were scaled to plant size (leaf mass proportion, rhizome mass proportion, root mass proportion, SLA, LAR, shallow root ratio, root and rhizome density) were analysed with ANOVAS. Growth rate (change in leaf area and leaf number) was analysed as a split plot repeated measures ANCOVA, with a first order autoregressive covariance structure and initial rhizome mass as the covariate. Fitness components (total biomass, ramets and flowers) were all measures related to plant size, and were analysed as ANCOVAS, using initial rhizome mass as the covariate. Each plant was treated as an independent experimental unit for class level analyses. Means comparisons among classes were made with independent contrasts. Proportional data were arcsine square root transformed before they were analysed. Data that did not meet the normality requirement of ANOVA were square root transformed.

Individual genotypes

Each genotypic class contained five genotypes represented by six replicate ramets, three in each water treatment. The ability to replicate individual genotypes clonally allowed us to compare trait expression among all 25 genotypes in both water treatments. A hybrid genotype was considered to have transgressive trait expression when it was significantly different from all 10 IB and IF genotypes in independent contrasts. The same data analysed for class level comparisons were analysed here with genotype, rather than genotypic class, as a main effect in the analyses. Analyses at the individual genotype level mirror those at the genotypic class level, and are meant to provide a contrast between group averages and individual differences. Rhizome tissue density data were not analysed at the genotype level due to lack of replication.

Results

CLASS-LEVEL ANALYSES

All physiological and growth traits measured at the end of this study significantly differed among genotypic classes (Table 1). Growth rate estimates from changes in leaf area throughout the study also showed significant differences among classes over time (class × time, $P < 0.001$, $F = 3.19$, n.d.f. = 20, d.d.f. = 811). Growth curves were linear, and differences between classes were consistent over time (leaf area at final harvest is shown in Table 2 as a representative example of differences between classes).

Among physiological traits, there was a significant main effect of water only on root mass proportion and shallow root ratio (Table 1). In flooded conditions, plants had a larger proportion of their biomass in root tissue, and a greater proportion of those roots allocated in the top layers of soil (Table 2). Rhizome mass proportion was the only trait that showed genotypic classes responding differently to water treatments (Fig. 1). Both IB and IF as well as BCIB hybrids produced a greater proportion of their biomass as rhizome tissue in the flooded treatment, while BCIF and F_1 hybrids produced a smaller proportion of their biomass as rhizomes (Fig. 1).

When no significant main effect of water was detected, physiological trait expression was compared among genotypic classes by calculating an average across both water environments (Table 2). Root mass proportion and shallow root ratio showed significant main effects of the water treatment, therefore expression of these two traits was compared in each environment separately. Comparison of genotypic classes revealed transgressive trait expression by the BCIB hybrid class for

Table 1 Analysis of physiological traits in five genotypic classes of Louisiana irises: *Iris brevicaulis*, *I. fulva*, F_1 hybrids, and first generation backcross hybrids towards each parent species. Plants were grown in two water treatments, wet and flood, for one growing season. Data were analysed as ANOVAS in SAS PROC MIXED. For all traits measured, degrees of freedom used for the *F*-test were the same (class d.f. = 4/112, water d.f. = 1/14, class × water d.f. = 4/112). Random effects (block and block × water) were not significant. Effects that are significant at $P \leq 0.05$ are shown in bold

	Class		Water		Class × water	
Trait measured (units)	*F*	*P*	*F*	*P*	*F*	*P*
Leaf mass proportion (g g^{-1})	6.51	**< 0.001**	0.12	0.738	1.81	0.131
Rhizome mass proportion (g g^{-1})	3.49	**0.010**	1.18	0.296	2.49	**0.048**
Root mass proportion (g g^{-1})	8.34	**< 0.001**	21.4	**< 0.001**	0.83	0.508
Leaf area ratio (m^2 g^{-1})	5.48	**< 0.001**	0.01	0.940	1.20	0.314
Specific leaf area (m^2 g^{-1})	4.72	**0.002**	0.26	0.619	0.62	0.652
Shallow root ratio (g g^{-1})	2.57	**0.042**	9.74	**0.008**	0.38	0.823
Root density (g mL^{-1})	2.61	**0.039**	3.94	0.067	1.27	0.285

Table 2 Means comparisons (LSMEANS ± SE generated by SAS PROC MIXED) of physiological traits and fitness components for five genotypic classes of Louisiana irises. Traits measured are listed on the left along with units of measurement. Superscript letters have been used to indicate differences between genotypic classes that are statistically significant. Hybrid classes that are significantly different from both parent species at $P \leq 0.05$ are shown in bold

	Genotypic class				
	Iris brevicaulis	BCIB	F_1	BCIF	*Iris fulva*
Physiological traits					
Leaf mass proportion (g g^{-1})	0.402 ± 0.015^{a}	0.312 ± 0.015^{b}	0.312 ± 0.015^{b}	0.348 ± 0.015^{b}	0.321 ± 0.015^{b}
Rhizome mass proportion (g g^{-1})	0.448 ± 0.013^{ab}	0.478 ± 0.013^{a}	0.458 ± 0.013^{ac}	0.433 ± 0.013^{bc}	0.415 ± 0.013^{b}
Root mass proportion (g g^{-1}), wet	0.127 + 0.010^{a}	0.141 + 0.010^{ab}	**0.155 + 0.010**^{b}	0.131 + 0.010^{a}	0.097 + 0.010^{c}
Root mass proportion (g g^{-1}), flood	0.145 + 0.010^{a}	0.163 + 0.010^{ab}	**0.191 + 0.010**^{b}	0.148 + 0.010^{a}	0.141 + 0.010^{c}
Leaf area ratio (cm^2 g^{-1})	5.63 ± 0.35^{a}	**3.48 ± 0.35**^{c}	5.34 ± 0.35^{a}	4.32 ± 0.35^{bc}	4.58 ± 0.35^{ab}
Specific leaf area (cm^2 g^{-1})	0.0140 ± 0.0014^{a}	0.0113 ± 0.0014^{a}	**0.0188 ± 0.0014**^{b}	0.0127 ± 0.0014^{a}	0.0143 ± 0.0014^{a}
Shallow root ratio (g g^{-1}), wet	0.36 + 0.03^{a}	0.42 + 0.03^{ab}	0.44 + 0.03^{b}	0.43 + 0.03^{ab}	0.42 + 0.03^{ab}
Shallow root ratio (g g^{-1}), flood	0.43 + 0.03^{a}	0.50 + 0.03^{b}	0.45 + 0.03^{ab}	0.50 + 0.03^{b}	0.47 + 0.03^{ab}
Root density (cm^3 g^{-1})	0.790 ± 0.015^{a}	0.797 ± 0.015^{a}	0.742 ± 0.015^{c}	0.755 ± 0.015^{abc}	0.751 ± 0.015^{bc}
Leaf area (cm^2)	1393.0 ± 199.7^{a}	2102.0 ± 196.2^{b}	**2817.73 ± 194.1**^{c}	2123.1 ± 194.6^{b}	1861.07 ± 195.9^{b}
Fitness components					
Total biomass (g), wet	65.86 + 8.64^{a}	**115.66 + 8.66**^{b}	**120.02 + 8.63**^{b}	**99.82 + 8.63**^{b}	75.07 + 8.80^{a}
Total biomass (g), flood	61.07 + 8.72^{a}	**92.45 + 8.64**^{b}	**92.49 + 8.68**^{b}	79.36 + 8.63^{ab}	60.23 + 8.79^{a}
Ramets (#), wet	4.99 + 0.54^{a}	3.99 + 0.54^{a}	7.27 + 0.54^{b}	4.98 + 0.53^{a}	5.71 + 0.54^{ab}
Ramets (#), flood	4.12 + 0.54^{ab}	3.53 + 0.54^{a}	5.49 + 0.54^{b}	4.42 + 0.53^{ab}	4.23 + 0.54^{ab}
Flower stalks (#)	0.67 + 0.22^{a}	0.82 + 0.22^{a}	0.65 + 0.22^{a}	1.14 + 0.22^{a}	0.72 + 0.23^{a}
Flowers (#)	0.91 + 1.15^{a}	5.34 + 1.30^{b}	4.02 + 1.21^{ab}	5.80 + 1.08^{b}	7.03 + 1.26^{b}

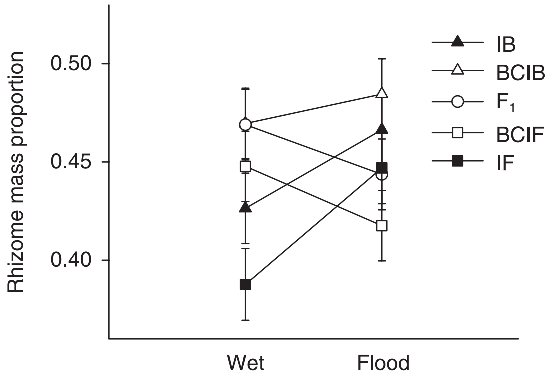

Fig. 1 Rhizome mass proportion means (shown as LSMEANS + SE computed with SAS PROC MIXED) for five genotypic classes of Louisiana iris: *Iris brevicaulis* (IB), *I. fulva* (IF), F_1 hybrids (F_1), and first generation backcross hybrids towards each parent species (BCIB and BCIF). Data are presented as reaction norms to show plant trait values in two water treatments, wet and flood. Rhizome mass proportion = rhizome biomass (g)/total biomass (g), calculated from dry biomass at the end of one growing season.

LAR and by the F_1 hybrid class for root mass proportion, SLA and final leaf area (Table 2). In all other cases, hybrid physiological traits were not statistically different from at least one parental species. In no case were hybrid physiological traits expressed in a way that was intermediate to the parent species. When the two species differed significantly in expression of a physiological trait, hybrids were equally likely to be similar to IB or IF (Table 2).

Both clonal fitness components (biomass and ramet production) were significantly different among genotypic classes (Table 3). Production of flower stalks was not affected by any of the terms in the model but, among plants that produced stalks, there were significant differences in number of flowers produced among genotypic classes (Table 3). Of the fitness components, only total biomass showed any sign of transgressive expression by hybrids (Table 2).

Water treatments significantly affected the production of biomass as well as ramets (Table 3). Plants produced smaller biomass and fewer ramets in the flood treatment (Fig. 2), suggesting that this water treatment was stressful for these plants. There were no statistically significant interactions between water treatment and genotypic class (Table 3), lending no support to the idea that relative clonal fitness of these genotypic classes differs between water treatments. Comparison of transgressive trait expression and fitness at the genotypic class level showed that all three hybrid classes had extremely high expression of the biomass fitness component (Table 2). However, regression analyses of physiological traits and fitness do not reveal any links between transgressive hybrids and high fitness (data not shown).

There were no significant differences among genotypes for the production of flower stalks (Table 3). However, when only plants that produced stalks were considered, there were significant differences among genoytypic classes in the number of flowers produced (Table 3). All hybrid genotypic classes and IF produced more flowers than IB (Table 2), suggesting that IB may have a lower level of sexual fitness than the other four groups.

INDIVIDUAL GENOTYPE-LEVEL ANALYSES

There were significant differences among genotypes for leaf mass proportion, root mass proportion, LAR,

Table 3 Analysis of fitness components measured on five genotypic classes of Louisiana iris. Total biomass and ramet (= rhizome) number are components of clonal fitness, whereas flower number is a component of sexual fitness. Data were analysed with ANCOVAs in SAS PROC MIXED, with initial rhizome biomass as the covariate. For total biomass, ramets and flower stalks, degrees of freedom used in the *F*-tests were the same (covariate d.f. = 1/45, class d.f. = 4/45, water d.f. = 1/14, class × water d.f. = 4/45). For flower analyses, degrees of freedom were slightly different from other analyses (covariate d.f. = 1/111, class d.f. = 4/111, water d.f. = 1/14, class × water d.f. = 4/111). Effects significant at $P \leq 0.05$ are in bold

	Covariate		Class		Water		Class × water	
Fitness trait	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Total biomass (g)	38.7	**< 0.001**	10.3	**< 0.001**	9.53	**0.008**	0.49	0.746
Ramets (#)	45.6	**< 0.001**	5.72	**< 0.001**	8.38	**0.012**	0.51	0.729
Flower stalks (#)	0.88	0.35	0.48	0.748	2.95	0.108	0.60	0.662
Flowers* (#)	7.27	**0.010**	4.26	**0.005**	4.11	0.062	2.11	0.095

*Analysis of flowers performed only on plants that produced flowering stalks.

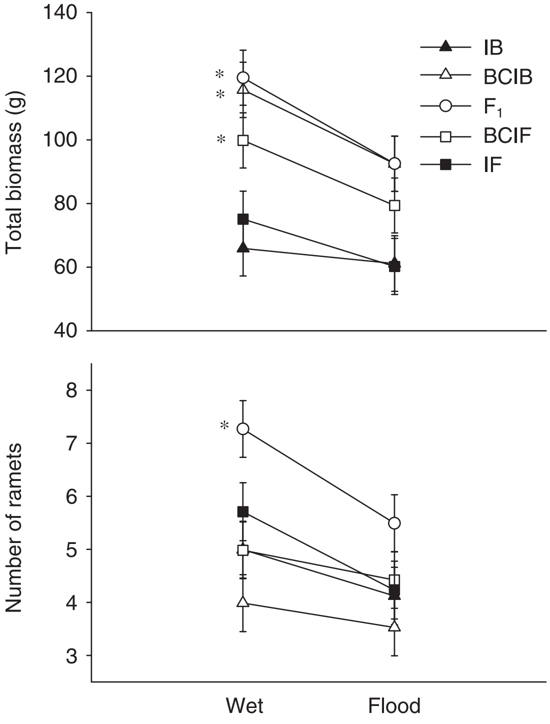

Fig. 2 Clonal fitness component means for five genotypic classes of Louisiana iris. Hybrid means that have extreme trait values that are significantly different from both parent species within a treatment in an independent contrast ($P \leq 0.05$) are indicated with an asterisk.

SLA and shallow root ratio, but not for rhizome mass proportion and root density (Table 4). Growth rate estimates based on leaf area changes were significantly different among genotypes over time (class × time, $P < 0.001$, $F = 2.5$, n.d.f. = 120, d.d.f. = 571). Several hybrid genotypes grew faster than either parent species on average, but no genotype was significantly different from all parental genotypes in means comparisons. (Final leaf area measured for growth rate analysis mirrored total biomass data, so growth rate data are not shown.)

At the individual genotype level, the only physiological trait affected by water treatments was root mass proportion (Table 4), with a larger proportion of total biomass produced as root tissue in the flood treatment (LSMEANS ± SE = 0.130 ± 0.003 in wet and 0.158 ± 0.003 in flood). There was, however, a significant effect of water treatment on growth rate estimates (water, $P = 0.026$, $F = 6.21$, n.d.f. = 1, d.d.f. = 14), with larger leaf areas produced in the wet treatment. There were no significant interactions between genotype and water for any physiological or growth traits.

Only two traits, SLA and shallow root ratio, showed signs of extreme or transgressive expression at the individual genotype level (Fig. 3). In each case, a single F_1 hybrid genotype (a different one for the two traits) had extremely high expression of the trait in the wet treatment. When means were compared between the

Table 4 Analysis of physiological traits in 25 genotypes of Louisiana irises. Each genotype was replicated clonally and subjected to both environmental treatments. Data were analysed with ANOVAs in SAS PROC MIXED. For all traits measured, degrees of freedom used for the *F*-test were the same (class d.f. = 24/72, water d.f. = 1/14, class × water d.f. = 24/72). Random effects block and block × water were not significant. Effects that are significant below $P \leq 0.05$ are shown in bold

	Genotype		Water		Genotype × water	
Trait measured (units)	*F*	*P*	*F*	*P*	*F*	*P*
Leaf mass proportion (g g^{-1})	3.61	**< 0.001**	0.14	0.711	1.13	0.339
Rhizome mass proportion (g g^{-1})	1.23	0.247	1.08	0.315	0.90	0.603
Root mass proportion (g g^{-1})	5.6	**< 0.001**	30.3	**< 0.001**	0.71	0.821
Leaf area ratio (m^2 g^{-1})	2.78	**< 0.001**	0	0.961	1.11	0.354
Specific leaf area (m^2 g^{-1})	2.25	**0.005**	0.74	0.404	1.64	0.057
Shallow root ratio (g g^{-1})	2.77	**< 0.001**	4.34	0.056	1.46	0.111
Root density (g ml^{-1})	1.43	0.127	4.25	0.058	1.04	0.434

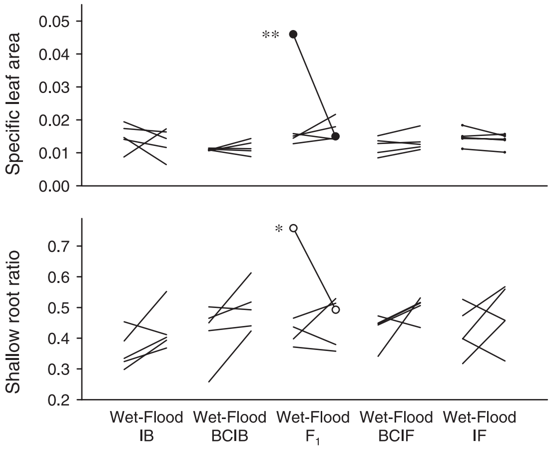

Fig. 3 Expression of specific leaf area (m^2 leaf area/kg leaf area) and shallow root ratio (root biomass in top half of pot/total root biomass) for 25 individual genotypes from five genotypic classes of Louisiana iris. One asterisk indicates a hybrid genotype by environment combination that is significantly different from eight of 10 IB and IF genotypes in that water treatment. Two asterisks indicates a hybrid genotype by environment combination that is significantly different from all 10 IB and IF genotypes in that water treatment.

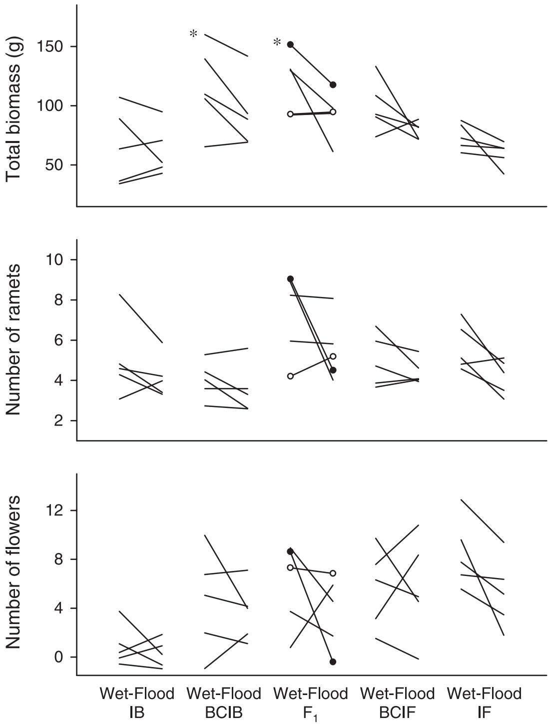

Fig. 4 Fitness components for individual genotypes of Louisiana iris. Total biomass and number of ramets are clonal fitness components, and number of flowers is a sexual fitness component. An asterisk indicates a hybrid genotype by environment combination that is significantly different from nine of 10 IB and IF genotypes in that water treatment. The two F_1 genotypes with extreme specific leaf area and shallow root ratio (shown in Fig. 3) are indicated by closed and open circle endpoints, respectively.

extreme hybrid genotype and the 10 genotypes of IB and IF within the wet treatment, 10 out of 10 comparisons were significant for SLA, and eight out of 10 comparisons are significantly different for shallow root ratio, suggesting a trend towards transgressive expression.

Both clonal fitness components were significantly lower in the flooded treatment overall, but flower stalk production did not differ among genotypes or water treatments (Table 5). Considering only plants that produced a flower stalk, only the covariate (initial rhizome weight) had a significant, slightly positive effect on the number of flowers produced. Variation among genotypes was extremely large for clonal fitness components and flowers produced (Fig. 4). Responses of some genotypes to water treatments were large, but not necessarily predictable, even within a genotypic class. No hybrid genotype was significantly larger than all IB and IF genotypes for any fitness component in either water treatment. For the biomass fitness component the largest BCIB and F_1 genotypes were significantly larger than nine out of 10 parental species genotypes in one or both water treatments (Fig. 4).

The link between transgressive trait expression and fitness was fairly weak at the genotype level. Only one of the two genotypes that displayed extreme SLA and shallow root ratio showed high levels of one fitness component (Fig. 4). The genotype with extreme root allocation patterns had modest levels of all fitness components. The genotype with extreme SLA had high levels of all fitness components in the wet treatment and very low levels of all fitness components in the flood treatment (Fig. 4). While these data only weakly

Table 5 Analysis of fitness components measured on 25 individual genotypes from five genotypic classes of Louisiana iris. Data were analysed with ANCOVAS in SAS PROC MIXED, with initial rhizome biomass as the covariate. For biomass, ramets and flower stalks, degrees of freedom used for the *F*-tests were the same (covariate d.f. = 1/71, genotype d.f. = 24/71, water d.f. = 1/14, genotype × water d.f. = 24/71). For analysis of flowers, degrees of freedom were slightly reduced (covariate d.f. = 1/11, genotype d.f. = 24/11, water d.f. = 1/12, genotype × water d.f. = 20/11). Effects that are significant below $P \leq 0.05$ are shown in bold

	Covariate		Genotype		Water		Genotype × water	
Fitness trait	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Biomass (g)	30.4	**< 0.001**	4.39	**< 0.001**	11.6	**0.004**	0.95	0.544
No. of ramets	33.5	**< 0.001**	2.67	**< 0.001**	9.36	**0.009**	0.83	0.689
No. of flower stalks	0.93	0.338	1.10	0.3705	2.62	0.128	1.52	0.089
No. of flowers	6.33	**0.029**	2.15	0.093	0.23	0.637	0.82	0.665

link transgressive traits and fitness, they do underscore the role of environmental context in fitness.

Discussion

Our data suggest that early generation Louisiana iris hybrids can possess novel phenotypic variation. We found evidence of transgressive expression of hybrid phenotypic traits and fitness components in two different environments both in genotypic classes and in individual genotypes. These results suggest that hybridization can generate a range of hybrid phenotypes, some of which have different ecological relationships from parent species. While genotypic class averages can reveal a tendency for an entire group of hybrids to differ from a parent species, one or a few extreme genotypes often seem to drive the pattern. To understand truly 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.

We recognize that extreme trait expression in an F_1 hybrid may be due to heterosis and, as a non-recombinant generation, there cannot, in fact, be transgressive segregation in the F_1s. Although formation of F_1 hybrids in Louisiana irises is fairly rare (Arnold *et al*. 1993), the high clonal fitness of F_1 hybrids suggests that this genotypic class can survive well and spread across many habitats. A single hybridization event may leave a deep mark on an iris population if F_1 hybrids persist and backcross (Hodges *et al*. 1996). Based on our data, it seems that extreme trait expression and high relative fitness of F_1 hybrids could play a role in generating hybrid novelty in the Louisiana iris system.

The transgressive expression of specific leaf area and shallow root allocation in some hybrid genotypes represents ecologically relevant physiological traits that appear to be the result of genetic recombination. Shallow 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 (Justin & Armstrong 1987; Ernst 1990; Armstrong *et al*. 1994). 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 conditions may allow it to survive better than individuals of either species in an environment that is shallowly flooded for extended periods of time. Specific leaf area may not relate to flood tolerance at all, but extremely high SLA would undoubtedly have an impact on the shade tolerance of the transgressive F_1 genotype (Poorter & Lambers 1986; Lambers *et al*. 1998). Traits related to shade tolerance would affect the amount of carbon fixed during a growing season, translating into rhizome mass or flower stalks. As extreme specific leaf area was only expressed in the wet treatment, it would suggest that this genotype might be best suited to conditions that are wet, but not flooded, and shady. The environment-dependent expression of a non-flood-related trait in a flooding experiment underscores the notion that transgressive expression of ecologically relevant traits may require a very particular (and sometimes unexpected) environment.

Hybrid fitness was high overall in the environmental conditions presented to plants in this experiment. All three hybrid classes were transgressive for total biomass in at least one treatment, suggesting that they can have higher clonal fitness than *I. brevicaulis* and *I. fulva* in some conditions. It seems likely that the clonal habit contributes to the success of these hybrids in the wild (Ellstrand *et al*. 1996), even if successful sexual reproduction is a rare event. Relative fitness of hybrid genotypic classes did not change between wet and flood treatments, indicating no environmental dependence of average hybrid fitness within a water gradient from waterlogged (wet) to shallow inundation (flood).

Fitness components measured at the genotypic class level revealed a potential difference in life history strategy between *I. brevicaulis* and *I. fulva.* While both clonal fitness components (Wikberg 1995) were relatively similar between species, *I. brevicaulis* plants flowered very little and *I. fulva* produced many flowers, suggesting that *I. fulva* has a more sexual reproductive strategy than *I. brevicaulis.* The most valuable hybrid novelty in terms of reproductive strategy may be in the combination of traits such as the ability to be both clonally and sexually prolific.

While some extreme hybrid traits were measured in this experiment, and many hybrids had high relative values of one or more fitness components, the link between the two was weak at best. Nearly all individuals with transgressive physiological and growth traits expressed them only in one of the two water treatments. Some of the most fit individual genotypes in one environment were among the least fit in the other. Our results did not show a clear relationship between physiological trait expression and fitness in the two environments tested. The actual fitness value of any individual trait can vary significantly by environment (Dudley 1996a). Traits such as root allocation to surface layers of soil may be advantageous while the soil is inundated, but become a liability when the water table drops (Ernst 1990). Certainly, the right habitat is critical for the expression and fitness advantage of novel hybrid traits. Wetlands, like many environments, contain a high degree of microhabitat heterogeneity (Snow & Vince 1984; Huenneke & Sharitz 1986) and are therefore rich in possibilities for genotype by environment interactions. Further exploration of the relationship between hybrid traits and hybrid fitness should involve direct phenotypic selection or similar analyses (Dudley 1996b).

This study found that there were detectable extreme hybrid phenotypes for ecologically important traits at both genotypic class and individual genotype levels. By averaging genotypic classes, some phenotypic variation was masked. Genotypic class averages were useful indicators of general trends in hybrid trait expression and fitness. If rare recombination events give rise to traits that might permit survival in extreme environments, leading to rapid ecological divergence (Rieseberg 1997; Rieseberg *et al*. 2003), it is the rare, transgressive phenotype that is most interesting. In the Louisiana iris system, we found evidence that some hybrid genotypes have extremely high values of one clonal fitness component, total biomass, and similar relative values to parental species for two other fitness components. If hybrids possess ecologically relevant novel traits, they will be capable of achieving high relative fitness if they arrive in the correct ecological context. By these means, successful creation and survival of novel hybrid genotypes can play a large role in the diversification and evolution of groups of species such as the Louisiana irises.

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 would also like to acknowledge C. Patterson, who spent many hours collecting data in the glasshouse. 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, E. Kentner, R. Wesselingh and three anonymous referees for comments on earlier versions of the manuscript. This research was supported by NSF grant DEB-0074159 to MLA.

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Received 23 October 2003
revision accepted 8 July 2004
Handling Editor: Joop Ouborg