

# Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana *Iris* hybrid population

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## Abstract

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

**Keywords:** environmental gradient, habitat selection, hybrid, hybrid novelty, Louisiana *Iris*, RAPDs

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## Introduction

Defining what makes a species ecologically unique can be a daunting challenge, due to the large number of factors that could be considered (Hutchinson 1957). In addition, species are most often defined by reproductive barriers (Dobzhansky 1937; Mayr 1942; Cracraft 1989; Templeton 1989). This may reveal little or nothing about functional or ecological differences. Phylogenetically related species often occupy similar niches within a community (e.g. Grace

& Wetzel 1982). However, if species occur in the same communities, traditional niche theory would predict that they must possess some ecological difference to coexist (Hutchinson 1959; but see Connell 1978 or Hubble & Foster 1986).

Rather than attempt to define all aspects of a niche, co-occurring species can be defined in terms of differences from their associates. In systems that are overwhelmingly influenced by a single strong abiotic gradient (e.g. flooding), this factor is the obvious choice to test for its effects on the ecological sorting of species (e.g. *Carex*, Moog 1998; *Ranunculus*, He *et al.* 1999). In more complex systems, it may be necessary to examine interactions of additional

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biotic or abiotic factors in addition to the primary gradient to explain niche separation and coexistence (Grace & Wetzel 1982; Hall & Harcombe 1998). In systems without an obvious gradient, it may be difficult to pinpoint individual factors that may be important in determining species distributions. The more factors and interactions that can be evaluated at one time, the more complete the understanding of the niche of the study species (Chapin *et al.* 1987).

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

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

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

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

The present study involves two species of Louisiana Iris, *Iris brevicaulis* and *I. fulva*. These two species are the most ecologically similar of the Louisiana Iris species, occurring at

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

To gain an understanding of important ecological differences between *I. brevicaulis* and *I. fulva*, we investigated the natural distributions within a hybrid population relative to environmental parameters. Within the general context of testing whether environment-dependent selection was occurring in the study population, we addressed the following questions.

- 1 Do *I. fulva* and *I. brevicaulis* occupy different types of habitat?
- 2 Are hybrids found in unique habitat relative to *I. brevicaulis* and *I. fulva*?
- 3 Is there a single environmental gradient that differentiates the habitats of each genotypic group?
- 4 Are individual genetic markers within hybrid genotypes associated with environmental factors?

## Materials and methods

### *Study population*

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

### *Environmental measurements*

It would be impossible to determine all the relevant biotic and abiotic variables influencing habitat distributions in Louisiana Irises. We chose to measure four abiotic factors that reflect conditions that affect a range of fundamental plant needs for every plant collected: elevation, percentage cover by canopy trees, soil pH and soil percentage organic matter. Three-dimensional co-ordinates were

measured with a TOPCON CRS-2 Total Station (Topcon America Corporation). A two-dimensional map of the individuals in the population was created using  $x$  and  $y$  co-ordinates. The  $z$  co-ordinate was used to determine elevation within the slough. Elevation was standardized to zero at the high water mark measured following rainfall in spring 1999.

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

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

### Molecular analysis

Nuclear and cytoplasmic DNA were isolated from each of the leaf samples using the procedures of Edwards *et al.* (1991). Seven diagnostic random nuclear markers for *I. fulva* and *I. brevicaulis* (Cruzan & Arnold (1993) and this study) were screened using random amplified polymorphic DNA (RAPD) methodology (Williams *et al.* 1990). Each marker was previously shown to be present in either *I. fulva* or *I. brevicaulis*, providing reliable data about the parentage of each individual. The polymerase chain reaction (PCR) amplification protocol for the RAPD analysis utilized one unit of Promega *Taq* polymerase, the Promega *Taq* reaction buffer, 1.9 mM MgCl<sub>2</sub>, 5 pmol of a single arbitrary primer and 2 µL of the stock DNA. Amplification was performed in an M. J. Research thermal cycler programmed for 45 cycles of 1 min at 92 °C, 1 min at 35 °C and 2 min at 72 °C. Primers used to generate markers came from the University of British Columbia Biotechnology Laboratory

(see Cruzan & Arnold 1993; except for UBC 502). The RAPD products were separated using 2% agarose gels containing ethidium bromide and then photographed under ultraviolet light.

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

### Statistical analyses

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

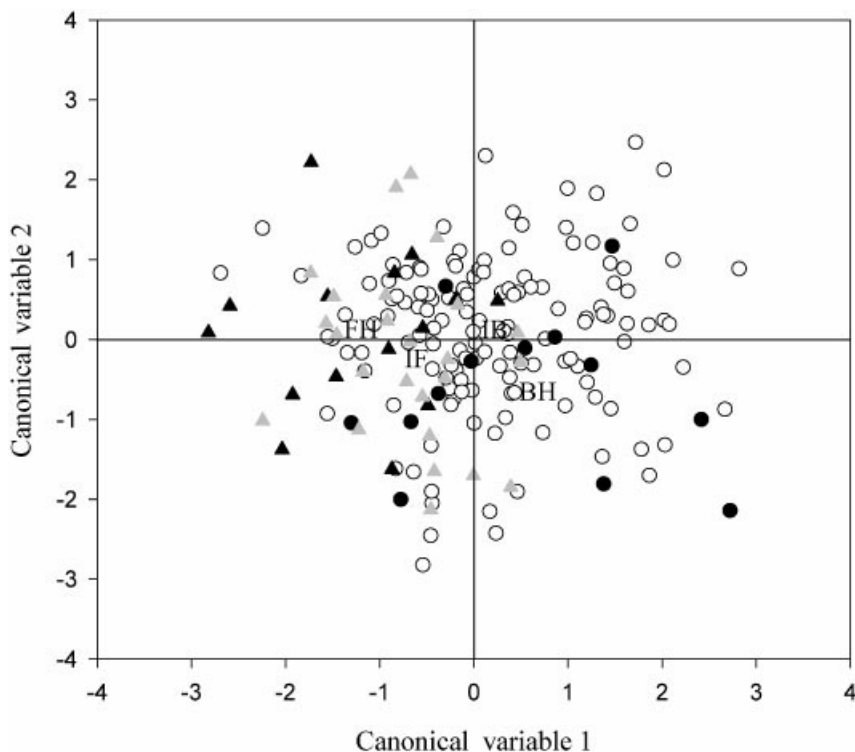
Prior to statistical analyses, the scale of each variable measured was standardized to become a dimensionless value falling between zero and one (Johnson 1998). This prevented interference by differences in units, magnitude, or dimension of variables. Ten data points were removed from the data set in order to satisfy the assumption for CDA that requires data to have a multivariate normal distribution. The 10 points removed fell two standard deviations beyond the mean of at least one variable and had a large skewing effect on the analysis. The most severe outliers were removed one at a time, until the discriminant functions stabilized. Removal of outlying data points generated a more conservative estimate of environmental influence than if all data points had been included in the analysis.

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

**Table 1** Genotypic class means of four environmental variables measured for canonical discriminant analysis of habitat preference in a Louisiana Iris hybrid zone

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

Values reported are means ± SD.



**Fig. 1** First and second canonical axes generated by canonical discriminant analysis of environmental associations in four genotypic classes of Louisiana Iris. Elevation, canopy cover, soil pH and soil organic matter were measured for each plant included in this analysis ( $n = 191$  plants). *Iris brevicaulis* and *I. brevicaulis*-like hybrids are represented by open and closed circles, respectively. *I. fulva* and *I. fulva*-like hybrids are represented by grey and black triangles, respectively. Means for each class are denoted by the following: IB, *I. brevicaulis*; BH, *I. brevicaulis*-like hybrids; FH, *I. fulva*-like hybrids and IF, *I. fulva*.

## Results

### Ecological associations of genotypic classes

Examining genotypic class means of environmental variables did not reveal any meaningful trends (Table 1). It was thus necessary to examine the results of the multivariate analysis to gain an understanding of how the genotypic classes differed in their habitat characterization.

Canonical discriminant analysis suggested the four genotypic classes occurred in somewhat different habitats (Fig. 1). *Iris brevicaulis* occupied the full range of both canonical variables, while the other three genotypic classes were more restricted in their distribution across habitat types. The means of each genotypic class (Fig. 1) showed separation

of *I. fulva* and *I. fulva*-like hybrids, from *I. brevicaulis* and *I. brevicaulis*-like hybrids along canonical variable 1 (Can1). Can2 further separated *I. brevicaulis*-like hybrids from the remaining classes. *Iris brevicaulis*-like hybrids and *I. fulva*-like hybrids were the most different groups as described by Can1 and Can2. Mahalanobis distance compared means of each pair of genotypic classes. *I. fulva* occurred in significantly different habitat compared to *I. brevicaulis* ( $P = 0.0006$ ). In addition, the hybrid classes occupied different habitats relative to one another ( $P = 0.0001$ ) and to the species they are least like genetically ( $P = 0.0025, 0.0001$ ; Table 2). Though not significant, there was also a trend for each species to be ecologically unique from its most similar hybrid.

The canonical discriminant analysis described 19% of the variation in the distributions of the four genotypic classes.

**Table 2** Probability that Mahalanobis distance indicates significant levels of habitat distinction between genotypic classes in a natural hybrid population of Louisiana Irises

Genotypic class	<i>Iris brevicaulis</i>	<i>I. brevicaulis</i> -like hybrids	<i>I. fulva</i> -like hybrids	<i>I. fulva</i>
<i>I. brevicaulis</i>	1.0000	0.0788 (n.s.)	0.0001***	0.0006***
<i>I. brevicaulis</i> -like hybrids		1.0000	0.0001***	0.0025**
<i>I. fulva</i> -like hybrids			1.0000	0.1166 (ns)
<i>I. fulva</i>				1.0000

*P*-values are shown for all pairwise comparisons. Statistical significance is indicated as follows: \*\*99%, \*\*\*99.9% and ns, not significant.

**Table 3** Canonical discriminant analysis for environmental associations in four genotypic classes of Louisiana Iris (*Iris brevicaulis*, *I. brevicaulis*-like hybrids, *I. fulva*-like hybrids, *I. fulva*)

Canonical variables	Unstandardized discriminant coefficients							Standardized discriminant coefficients			
	Elevation	Cover	Soil organics	Soil pH	Eigenvalue	% contrib.	<i>P</i> < 0.05	Elevation	Cover	Soil organics	Soil pH
Can1	5.907	-0.922	-0.921	-0.349	0.2370	77.48	0.0001	1.138	-0.200	-0.175	-0.071
Can2	-0.585	5.1859	1.987	-1.987	0.0422	13.80	0.0499	-0.113	1.047	0.378	-0.401
Can3	3.310	-2.055	4.700	-1.506	0.0267	9.73	0.0860	0.675	-0.418	0.894	-0.308

Discriminant coefficients describe the discriminant functions generated from all environmental variables to maximize separation between genotypic classes. The amount of variation each canonical variable describes is calculated from the eigenvalue. *P*-values show the probability that each canonical variable describes a significant amount of variation.

**Table 4** Correlation of canonical variables generated to describe habitat associations of a hybrid population of Louisiana Irises with each of four environmental variables

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

A higher absolute value for correlation denotes a larger contribution by that environmental variable.

Within the variation described by canonical analysis (Table 3), Can1 was responsible for 77% of the separation of these four groups. Can2 accounted for 14% of the separation. The third canonical variable did not explain a statistically significant amount of the separation. Each canonical variable was strongly correlated with only one of the environmental factors (Table 4). Can1 was tightly correlated with elevation ( $r = 0.977$ ), Can2 was associated with percentage canopy cover ( $r = 0.811$ ) and Can3 was dominated by percentage soil organic matter ( $r = 0.817$ ). Contrary to the simple means of each environmental factor, the canonical means for each genotypic class suggested that *I. brevicaulis* and *I. brevicaulis*-like hybrids did tend to live in higher

elevation sites than *I. fulva*, and especially *I. fulva*-like hybrids (Fig. 1). Sorting along the Can2 axis suggests that *I. brevicaulis*-like hybrids may grow in areas with slightly more open canopies than the other three groups. The hybrid classes lived in a similar range of habitats to *I. fulva*, and a slightly narrower range than *I. brevicaulis*.

#### Ecological associations of individual markers

When hybrid genotypes were analysed separately with canonical discriminant analysis, several individual markers correlated with unique environmental factors. This correlation is summarized by Can1 (Table 5). In contrast, three

**Table 5** Canonical discriminant analysis of environmental associations of individual RAPD loci in hybrid Louisiana Irises

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

Frequency of a given marker (Q), correlation of environmental factors with the canonical variable generated, probability that separation of groups is achieved, and the means of the canonical variable for the *Iris brevicaulis* (B) and *I. fulva* (F) forms of each marker are given. ns, not significant.

markers, L180, F165A and B502-2 did not show significant separation when multivariate discriminant analysis was performed. All remaining markers showed significant, but slightly different, associations with environmental variables. In all cases, elevation appeared to be the major factor. Soil percentage organic content and pH were moderately correlated with all canonical variables that described habitat differences. Canopy cover was the least important environmental factor in determining habitat preferences. The canonical variable constructed for each marker was slightly different. However, since all markers were similarly correlated with the environmental variables, means of canonical variables for the *I. brevicaulis* and *I. fulva* forms of the markers were also similar. The exception is F154, which had the most unique environmental associations. F154 had the strongest correlation with soil organic matter ( $r = -0.755$ ), and appeared to have an association with elevation that was the inverse of all other markers (Table 5).

## Discussion

### Genotypic classes and habitat associations

There were significant differences between the habitats occupied by *Iris brevicaulis* and *I. fulva*. On average, *I. fulva* occurred at lower elevations in deeper cover than *I. brevicaulis*, but with some overlap. These findings generally support previous descriptions of *I. brevicaulis* and *I. fulva* habitat differences (Viosca 1935; Cruzan & Arnold 1993). In this population, *I. brevicaulis* exists in a wide range of possible habitats. In contrast, *I. fulva* occupies a narrow range of habitat, as described by canonical variable 1 (Can1). Our analysis thus suggests that *I. fulva* is somehow restricted to lower elevation sites. Similar patterns have been observed in *Typha* and *Ranunculus* (Grace & Wetzel 1982; He *et al.*

1999), and can be explained by competitive displacement from the more optimal (i.e. drier) habitat. In these systems, higher flood tolerance in one species permits coexistence with another competitive dominant.

In the Louisiana Iris system it was assumed that the range for *I. brevicaulis* did not include areas under standing water due to low flood tolerance. The previously reported restriction of *I. fulva* to more flooded, presumably less optimal, habitat (Viosca 1935; Cruzan & Arnold 1993) could have been explained by higher flood tolerance in *I. fulva*, and weak competitive ability against *I. brevicaulis* on drier ground. Unexpectedly, the elevation data in the current study indicate that *I. brevicaulis* can live quite successfully in areas that are regularly under standing water. Possibly, *I. fulva* and *I. brevicaulis* have similar flooding tolerance, but *I. fulva* may not be tolerant of dry conditions, limiting it to lower elevation sites. We did not find sharp zonation between the two species, as was previously reported, and will address competitive ability specifically in future studies.

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

*I. brevicaulis*-like hybrids and *I. fulva*-like hybrids occupy the most distinct habitat types of any pairwise comparison.

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

Hybrid genotypes occupy a broad range of possible habitats, but seem largely to overlap with their closest parental species. Individual hybrid genotypes do occur on the periphery of the canonical distribution, suggesting that they could potentially occupy unique habitat types. Two of the 10 outlying points which were removed from the analysis were hybrid genotypes, the other eight were *I. brevicaulis*, demonstrating that hybrid genotypes are among those genotypes that occur in habitats that fall more than two standard deviations from the norm. Visual interpretation of the data suggests that *I. fulva* is more similar to *I. fulva*-like hybrids than *I. brevicaulis* is to *I. brevicaulis*-like hybrids. However, the apparent differences are not supported by the statistics (Table 2). In a previous study, across a more diverse range of habitat, Cruzan & Arnold (1993) found that *I. fulva*-like hybrids were statistically different from *I. fulva*, while *I. brevicaulis*-like hybrids were not significantly different from *I. brevicaulis*. The current study found that, on average, neither hybrid class occurred in habitat that was unique from its closest parent.

Interpretation of the means of these genotypic classes should include the caveat that the *I. brevicaulis*-like hybrid class encompasses more variation than the *I. fulva*-like hybrids. The *I. fulva*-like hybrids were comprised of two RAPD genotypes with one *I. brevicaulis* marker and seven *I. fulva* nuclear and cytoplasmic markers. It is likely that these individuals are advanced generation hybrids. The *I. brevicaulis*-like hybrids are made up of eight unique RAPD genotypes that contain one to four *I. fulva* markers, and therefore four to seven *I. brevicaulis* nuclear and cytoplasmic markers. There are *I. brevicaulis*-like individuals that represent potential early generation hybrids, one that may be an  $F_1$ . Therefore, differences in average response to environmental variables by hybrid classes may have been due, in part, to variation in genotypic diversity.

Ideally, there would be enough replication of each hybrid genotype to statistically analyse each one as its own class. However, several genotypes occur only once, prohibiting such an analysis. By grouping hybrids into two types, *I. brevicaulis*-like and *I. fulva*-like, we believe we have separated the individuals into biologically relevant groups.

All the members of a genotypic class share a cpDNA haplotype and the majority of their nuclear alleles. While having two classes of hybrids does not account for all of the variation that is likely found among hybrid individuals, we feel it is an improvement over one catch-all hybrid category commonly used in studies of natural hybrid populations (see Arnold & Hodges 1995; Arnold 1997; for a discussion and references).

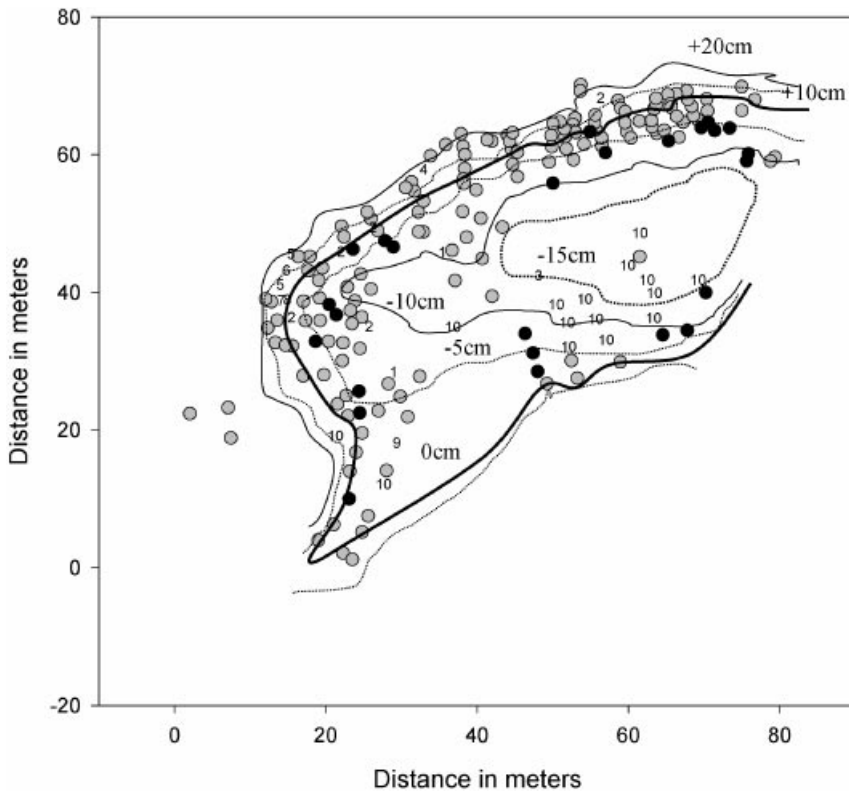
#### *Individual loci and habitat associations*

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

Clearly, our present results suggest an effect of environment-dependent selection. However, the pattern is not entirely consistent with the Mosaic model (Rand & Harrison 1989) because there is overlap in the distributions of the genotypic classes, rather than clusters that mirror habitat patchiness. The habitat in which we sampled might better be described by subtle environmental gradients overlaid by microsite differences, as opposed to a mosaic of well-defined habitat patches. In addition, because hybrids are scattered throughout the parental species' distributions (Fig. 2), they do not appear to be marginalized to ecotonal habitat (Moore 1977) as would be expected from the Bounded Hybrid Superiority model.

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

The current study was conducted on a very fine scale both spatially and in terms of the magnitude of environmental



**Fig. 2** Spatial distribution of Louisiana Iris genotypes in a natural population of *Iris brevicaulis*, *I. fulva*, and their hybrids. *I. brevicaulis* and *I. fulva* are represented by grey and black circles, respectively. Numbers denote unique hybrid genotypes. 1–8 are *I. brevicaulis*-like hybrids, 9 and 10 are *I. fulva*-like hybrid genotypes.

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

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The authors are interested in the evolutionary significance of natural hybridization. In particular, they are continuing their research into the origin and transfer of adaptations among hybridizing species. They are also currently exploring the evolutionary origin of hybrid species within the Louisiana Irises.

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