HYBRID FITNESS IN THE LOUISIANA IRISES: ANALYSIS OF PARENTAL AND F$_1$ PERFORMANCE

JOHN M. BURKE,[1] SHANNA E. CARNEY,[2] AND MICHAEL L. ARNOLD[3]
Department of Genetics, University of Georgia, Athens, Georgia 30602-7223
[1]*E-mail: burke@dogwood.botany.uga.edu*
[3]*E-mail: arnold@dogwood.botany.uga.edu*

Abstract.—The assumption of hybrid inferiority is central to the two models most widely applied to the prediction of hybrid zone evolution. Both the tension zone and mosaic models assume that natural selection acts against hybrids regardless of the environment in which they occur. To test this assumption, we investigated components of fitness in *Iris fulva, I. hexagona* and their reciprocal F$_1$ hybrids under greenhouse conditions. The four cross types were compared on the basis of seed germination, vegetative and clonal growth, and sexual reproduction. In all cases, the hybrids performed as well as, or significantly better than, both of their parents. These results suggest that F$_1$ hybrids between *I. fulva* and *I. hexagona* are at least as fit as their parents. The results of this study are therefore inconsistent with the assumptions of both the tension zone and mosaic models of hybrid zone evolution.

Key words.—Hybrid fitness, Louisiana irises, mosaic hybrid zones, natural hybridization, natural selection, tension zones.

The evolutionary importance of natural hybridization is a topic over which there has been considerable debate. The predominant viewpoint is that hybridization is evolutionarily unimportant because crosses between genetically divergent individuals often result in the production of offspring that are less fertile or less viable than those produced as a result of crosses between genetically similar individuals (e.g., Mayr 1963, 1992; Wagner 1970). A less commonly held opinion is that natural hybridization is a relatively widespread and potentially creative evolutionary process (e.g., Anderson 1949; Anderson and Stebbins 1954; Lewontin and Birch 1966; Dowling and DeMarais 1993; B. R. Grant and Grant 1993; Arnold 1997). Indeed, introgression (the transfer of genetic material from one species into another through repeated backcrossing) has been documented in a wide variety of both plant and animal taxa (Arnold 1992; Rieseberg and Wendel 1993). In addition, there is evidence that introgressive hybridization may serve as a source of adaptive genetic variation (Lewontin and Birch 1966; P. R. Grant and Grant 1994; B. R. Grant and Grant 1996; Wang et al. 1997).

The assumption of hybrid inferiority lies at the heart of the two models most widely applied to the prediction of hybrid zone evolution. The first model, known as the tension zone or dynamic equilibrium model (Key 1968; Barton and Hewitt 1985), assumes that the stability of hybrid zones is maintained by a balance between selection against hybrid individuals and the continual dispersal of parental individuals into the zone of contact. The second model, known as the mosaic model (Harrison 1986; Howard 1986), also assumes that hybrids are uniformly unfit. The distribution of parental genotypes under the mosaic model is, however, governed by environment-dependent selection. This distribution reflects the adaptation of the parental genotypes to different, patchily distributed habitats. Although the tension zone and mosaic models are based on environment-independent and environment-dependent selection, respectively, it is important to note that, in both cases, selection against hybrids is assumed to be uniform across all environments.

While hybrid inferiority is central to most predictions of hybrid zone evolution, it is an assumption that has been largely untested. However, a review of studies that estimated the fitness of plant and animal hybrids found that the majority of hybrid classes did not demonstrate the lowest fitness (Arnold and Hodges 1995). In fact, hybrid genotypes were found to be less fit than their parents in only 10 of 37 comparisons. In contrast, hybrids were at least equivalent to, if not more fit than, both of their parental taxa in 20 of the 37 comparisons and were intermediate to their parents in the remaining seven comparisons. This led the authors to conclude that, in general, hybrid individuals are not uniformly unfit. Rather, they may exhibit a variety of fitnesses, ranging from less fit to more fit than their parents. In the present study, we examined the fitness of hybrid and parental genotypes of Louisiana irises under experimental conditions.

Iris fulva Ker-Gawler and *I. hexagona* Walter (Iridaceae) are members of the Louisiana iris species complex. *Iris fulva* is generally associated with shady understory habitats along the banks of bayous of the Mississippi River, whereas *I. hexagona* tends to occur in open, freshwater marshes and swamps across the southeastern United States (Viosca 1935; Bennett 1989). Although both of these species have relatively wide ranges, they occur sympatrically in southern Louisiana. While interspecific matings have led to the production of numerous hybrid populations throughout the area of overlap (Viosca 1935; Anderson 1949; Arnold et al. 1990a,b, 1991), F$_1$ seed formation is rare (Arnold et al. 1993) and analyses of several hybrid *Iris* populations have failed to detect the presence of any adult F$_1$ hybrids (Arnold et al. 1990a,b, 1991, 1992; Nason et al. 1992; Arnold 1993). These findings suggest that F$_1$ hybrids between *I. fulva* and *I. hexagona* are relatively less fit than their parents.

A previous study, which involved reciprocally transplanting *I. fulva, I. hexagona,* and F$_1$ and F$_2$ hybrids, indicated

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[2] Present address: Department of Biology, Indiana University, Bloomington, Indiana 47405; E-mail: scarney@sunflower.bio.indiana.edu.

TABLE 1. ANCOVAs of the effect of cross type, fruit half, seed coat removal, and seed weight on percent germination, weeks to germination, and total leaf area nine and 18 weeks after germination. Significant P-values ($P \leq 0.05$) are indicated with bold type.

Source	Percent germination			Weeks to germination			Leaf area at 9 weeks			Leaf area at 18 weeks		
	df	MS	P	df	MS	P	df	MS	P	df	MS	P
Cross type	3	0.11	0.1955	3	57.27	**0.0001**	3	65,904	**0.0001**	3	925,073	**0.0003**
Fruit half	1	0.02	0.5960	1	0.10	0.8135	1	6816	0.2938	1	63,878	0.4964
Seed coat	1	0.60	**0.0048**	1	0.15	0.7782	1	1222	0.6559	1	657	0.9450
Cross × half	3	0.10	0.2366	3	1.17	0.5955	3	5407	0.4524	3	72,138	0.6653
Cross × coat	3	0.12	0.1646	3	1.07	0.6311	3	6865	0.3440	3	110,889	0.4919
Half × coat	1	0.01	0.7987	1	0.41	0.6403	1	247	0.8412	1	405	0.9568
Weight	1	0.18	0.1154	1	0.01	0.9530	1	645	0.7461	1	11,220	0.7754
Error	100	0.07		100	1.85		100	6119		100	137,098	

that once their rhizomes are established, hybrids between these species can survive and reproduce clonally at least as well as their parents in all habitats studied, and can outperform their parents in some habitats (Emms and Arnold 1997). Because the transplants were performed with rhizome material, however, this study did not address hybrid fitness during early life-history stages and may have missed selection acting against the hybrids during the seed-to-seedling and seedling-to-adult transitions. The goal of the present study was to estimate the relative fitnesses of *I. fulva, I. hexagona*, and their reciprocal F_1 hybrids under greenhouse conditions from the seed-to-adult stages. To this end, we examined differences in (1) seed germination; (2) vegetative/clonal growth from the seedling stage to sexual maturity; and (3) sexual reproduction. This analysis of the fitness of F_1 hybrid and parental individuals allowed us to test the common assumption (i.e., uniform hybrid unfitness) of the two models most frequently used to predict the evolutionary outcome of natural hybridization.

MATERIALS AND METHODS

Seed Production and Germination

The seeds used in this study were produced in the crosses described by Carney et al. (1996). Pure conspecific and heterospecific pollinations were performed on flowers of both *I. fulva* and *I. hexagona* to produce parental and reciprocal F_1 hybrid seeds. All crosses were carried out between 24 March and 31 March 1994 in a natural *Iris* population located 7.3 km west of Labadieville in Assumption Parish, Louisiana. Each day, pollinations were performed with mixtures consisting of pollen collected from nine to 40 randomly selected *I. fulva* individuals (23.33 ± 4.07 individuals, mean ± SE) or five to 24 randomly selected *I. hexagona* individuals (14.80 ± 3.73 individuals). Since pollen carryover is common in many species, including the Louisiana irises, and often occurs at high levels (Schaal 1980; Thomson and Plowright 1980; Price and Waser 1982; Waser and Price 1984; Hodges et al. 1996), it is likely that mixed pollen loads are a regular occurrence in natural populations of *I. fulva* and *I. hexagona*. The pollen loads used in this study are, therefore, similar to the expected composition of natural pollen loads.

Throughout this paper, F_1 hybrid seeds produced as a result of pollinating *I. fulva* flowers with *I. hexagona* pollen will be referred to as IF × IH seeds, whereas those resulting from the reciprocal cross will be referred to as IH × IF seeds. One

fruit was randomly selected from each of nine, five, six, and nine different maternal individuals from the *I. fulva*, IF × IH, IH × IF, and *I. hexagona* cross types, respectively. The location of all seeds within each of these fruits was recorded. Identification of ovule position is possible in *Iris* because unfertilized and aborted ovules are visible within the mature ovary (Carney et al. 1996). Because position specific fitness differences have been documented in several other studies (e.g., Stephenson et al. 1988; Rocha and Stephenson 1990), seeds were assigned to the stylar (top) or peduncular (bottom) halves of each fruit. This allowed us to test for fitness differences between fruit halves.

Prior to planting, each seed was weighed. To determine the weight of the embryo and endosperm alone, half of the seeds from each fruit were then randomly selected to have their corky outer seed coat removed and were reweighed. Seeds from each of the cross type × fruit half × seed coat treatment classes were then planted in 48 cell flats in a completely randomized design. Flats were arranged on a single bench in the Botany Department greenhouse at the University of Georgia. Seed germination was recorded during weekly censuses from 17 July 1995 to 1 January 1996.

Growth and Reproduction

Length and width of the longest leaf and the number of leaves produced were recorded at nine and 18 weeks after germination. Total leaf area per individual was then estimated by multiplying length by width by leaf number. Eighteen weeks after germination, seedlings were randomly selected for repotting into six-inch pots. The seedlings were first divided into three date classes (early, middle, and late) based on their germination date. Within the middle and late date classes, 19 seedlings were then chosen at random from each cross type × fruit half treatment class. The early date class was not included, as it did not contain individuals from all of the cross type × fruit half treatment classes.

Date of first flower and the total number of flowers produced were recorded for each of the repotted plants. Each flower was then pollinated with a mixture of parental and F_1 hybrid pollen. Whenever possible, pollen from newly dehisced anthers was used, and pollen more than 24 hours old was not used. The resulting fruits were collected at maturity and the total number of fruits and seeds produced per plant, as well as percent fruit set (i.e., the percent of pollinated flowers that produced a mature fruit) were recorded.

TABLE 2. Rank order of fitness components within each of the four life-history stages for *I. fulva*, IF × IH, IH × IF, and *I. hexagona*. Inequalities reflect statistical significance (*P* ≤ 0.05) as indicated by Tukey's studentized range test. Although *I. hexagona* was not included in the analyses of the flowering data (because only one of 76 individuals flowered), it was included parenthetically in this table to indicate that it had obviously lower levels of sexual reproduction than the other three cross types.

Fitness component	Rank order of fitness
Germination	
Percent germination	*I. fulva* = IH × IF = IF × IH = *I. hexagona*
Weeks to germination	IH × IF = *I. fulva* > IF × IH = *I. hexagona*
Growth	
Leaf area at 9 weeks	IH × IF = *I. hexagona* = IF × IH > *I. fulva*
Leaf area at 18 weeks	IH × IF ≥ IF × IH = *I. hexagona* = *I. fulva*
Leaf area at 38 weeks	IH × IF ≥ IF × IH = *I. fulva* = *I. hexagona*
Clonal reproduction	
Number of ramets	*I. hexagona* = IH × IF = IF × IH > *I. fulva*
Ramet mass	IH × IF = *I. hexagona* = IF × IH > *I. fulva*
Sexual reproduction	
Percent flowering	*I. fulva* ≥ IH × IF = IF × IH (≫*I. hexagona*)
Weeks to first flower	IH × IF ≥ IF × IH = *I. fulva*
Number of flowers	*I. fulva* ≥ IF × IH = IH × IF (≫*I. hexagona*)
Number of seeds	IF × IH = IH × IF = *I. fulva* (≫*I. hexagona*)
Percent fruit set	IH × IF ≥ IF × IH = *I. fulva*.

All plants were harvested 38 weeks after germination and measurements of growth and asexual reproduction were recorded. Specifically, the number of ramets (distinct rhizome branches) per plant, total ramet mass, the number of leaves per ramet, and the length of the longest leaf on each ramet were recorded. The width of the longest leaf was estimated from length-width regressions, and the total leaf area produced per plant was estimated by multiplying length by estimated width by number of leaves on each ramet and summing over ramets.

Statistical Analyses

The data were insufficiently balanced to test for maternal effects. Therefore, to prevent pseudoreplication (i.e., treating multiple seeds from a single maternal individual as if they were statistically independent), we analyzed mean values for the seeds from each maternal individual. Furthermore, because multiple crosses were carried out on any given day, some of the fruits in this study were produced by crosses with the same pollen mixtures. It is therefore possible that seeds from different maternal individuals were pseudoreplicates. The large number of pollen donors included in each mix, however, would tend to minimize the amount of pseudoreplication. In addition, reanalysis of the data using only those fruits that were produced with different pollen mixtures revealed the same overall pattern of performance in all 12 of the traits measured. The main difference between the two sets of analyses was a decrease in statistical power in the reanalysis relative to the full analysis due to a decrease in sample sizes. Because both analyses lead to the same overall conclusions, we have reported only the results from the full dataset in this paper.

Total seed weight as well as the proportion of total seed weight accounted for by the embryo and endosperm (i.e., not including the corky outer seed coat) were analyzed as two-factor ANOVAs with cross type and fruit half as main effects. Percent germination, mean number of weeks to germination, and mean

leaf area after nine and 18 weeks were analyzed as three-factor ANCOVAs with cross type, fruit half, and seed coat as fixed effects and seed weight as a covariate. Mean number of ramets produced, mean ramet mass, leaf area after 38 weeks, percent flowering, mean number of weeks from germination to first flower, mean number of flowers produced, percent fruit set, and mean number of seeds produced per flowering individual were analyzed as three-factor ANCOVAs with cross type, fruit half, and date class as fixed effects and seed weight as a covariate. All main effects and interactions were tested over the residual error. Percent germination, percent flowering, and percent fruit set were arcsine transformed prior to analysis. A posteriori comparisons were made using Tukey's studentized range test, which
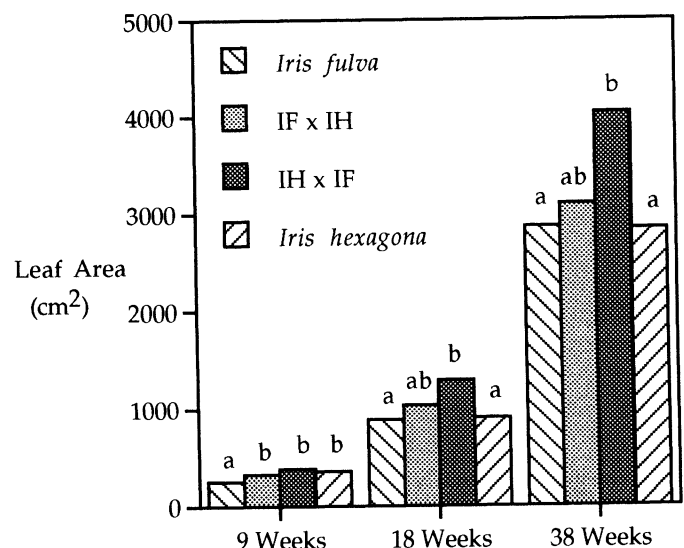

FIG. 1. Mean leaf area nine, 18, and 38 weeks after germination for *Iris fulva*, IF × IH, IH × IF, and *I. hexagona* individuals. Within a time period, cross types sharing a letter are not significantly different (*P* ≤ 0.05) as indicated by Tukey's studentized range test. See text for standard error values.

TABLE 3. ANCOVAs of the effect of cross type, fruit half, date class, and seed weight on total leaf area 38 weeks after germination, the number of ramets produced per individual, ramet mass per individual, percent flowering, number of weeks to first flower, number of flowers produced, number of seeds produced per flowering individual, and percent fruit set. Significant P-values ($P \leq 0.05$) are indicated with bold type.

Source	Leaf area at 38 weeks			Number of ramets			Ramet mass			Percent flowering		
	df	MS	P	df	MS	P	df	MS	P	df	MS	P
Cross type	3	7,214,200	**0.0024**	3	24.70	**0.0339**	3	6555	**0.0002**	2	0.94	**0.0188**
Fruit half	1	367,546	0.6082	1	4.83	0.4444	1	751	0.3677	1	0.08	0.5449
Date class	1	35,965,626	**0.0001**	1	55.39	**0.0108**	1	10,792	**0.0009**	1	2.37	**0.0018**
Cross × half	3	655,066	0.7027	3	8.12	0.3998	3	176	0.9011	2	0.12	0.5760
Cross × date	3	325,510	0.8721	3	51.16	**0.0007**	3	3561	**0.0116**	2	1.20	**0.0068**
Half × date	1	566,964	0.5244	1	1.01	0.7259	1	969	0.3064	1	0.30	0.2471
Weight	1	76,574	0.8149	1	14.78	0.1822	1	65	0.7904	1	0.04	0.6891
Error	88	1,388,193		88	8.17		88	916		59	0.22	

controls the Type I experimentwise error rate ($\alpha = 0.05$). All analyses were performed using SAS (SAS Institute 1990).

RESULTS

Seed Weight

While the effect of cross type on mean seed weight was significant ($F_{3,50} = 36.03$, $P \leq 0.0001$), the effects of both fruit half and the cross × half interaction were nonsignificant ($F_{1,50} = 0.61$ and $F_{3,50} = 0.29$, both $P \geq 0.44$). The *I. hexagona* cross produced significantly heavier seeds (140.6 ± 5.44 mg, mean $\pm$ SE) than the other three cross types, and the IH × IF cross produced significantly heavier seeds (119.6 ± 4.51 mg) than the remaining two cross types. The *I. fulva* and IF × IH crosses produced seeds that were not significantly different from each other (82.8 ± 5.53 mg and 68.8 ± 4.08 mg, respectively). Analysis of the seed weight ratio data revealed that the effects of cross type ($F_{3,50} = 0.19$, $P = 0.90$), fruit half ($F_{1,50} = 0.16$, $P = 0.69$), and their interaction ($F_{3,50} = 0.30$, $P = 0.82$) on the proportion of the total seed weight that is accounted for by the endosperm and embryo were all nonsignificant. This result indicates that the four cross types produced seeds with the same pattern of resource allocation between the embryo/endosperm and seed coat and, therefore, that total seed weight is an accurate representation of the weight of the embryo and endosperm in seeds from all four cross types.

Germination and Survival

Mean percent germination did not vary significantly among cross types or fruit halves (Tables 1, 2). *Iris fulva* seeds exhibited the highest percent germination ($87.51 \pm 3.52\%$, least squares mean $\pm$ SE), followed by IH × IF ($79.52 \pm 3.91\%$), IF × IH ($78.07 \pm 5.07\%$), and *I. hexagona* seeds ($75.82 \pm 4.22\%$). Although the effect of seed coat removal on percent germination was significant ($P = 0.0048$; coat removal caused an overall mean decrease in percent germination), the cross type × coat interaction was nonsignificant, indicating that the removal of the seed coat did not have a differential effect on the germination rate of seeds from the four cross types. All other effects were nonsignificant.

Cross type had a highly significant effect on the number of weeks between planting and germination ($P \leq 0.0001$; Tables 1, 2). On average, the IH × IF and *I. fulva* seeds germinated significantly faster (23.00 ± 0.30 weeks and 23.03 ± 0.27 weeks, respectively) than the IF × IH and *I. hexagona* seeds (24.96 ± 0.39 weeks and 26.00 ± 0.32 weeks, respectively). All other effects were nonsignificant. Survival rate from germination to the conclusion of the study was high for all cross types. Only one individual from each of the *I. fulva*, IF × IH and *I. hexagona* cross types died during the study, and no IH × IF individuals died.

Growth and Clonal Reproduction

The effect of cross type on total leaf production per plant at nine and 18 weeks after germination was highly significant ($P \leq 0.0001$ and $P = 0.0003$, respectively; Tables 1, 2; Fig. 1). IH × IF, *I. hexagona* and IF × IH plants produced significantly more leaf area nine weeks after germination (374.83 ± 17.20 cm^2, 350.80 ± 18.57 cm^2, and 319.74 ± 22.32 cm^2, respectively) than did *I. fulva* plants (246.85 ± 15.48 cm^2). Furthermore, IH × IF plants produced significantly more leaf area 18 weeks after germination (1295.84 ± 81.44 cm^2) than *I. hexagona* (906.18 ± 87.89 cm^2) and *I. fulva* plants (887.87 ± 73.25 cm^2). IF × IH leaf production at this time point was not significantly different from that of the other three cross types (1036.29 ± 81.44 cm^2). All other effects were nonsignificant at both nine and 18 weeks after germination. A similar result was obtained in the analysis of total leaf area 38 weeks after germination (Tables 2, 3; Fig. 1). Again, the effect of cross type was significant ($P = 0.0024$), with IH × IF plants producing significantly more leaf area (4036.49 ± 272.86 cm^2) than *I. fulva* (2862.94 ± 252.07 cm^2) and *I. hexagona* plants (2842.87 ± 285.96 cm^2). IF × IH leaf production at 38 weeks was not significantly different from that of the other three cross types (3099.26 ± 350.13 cm^2). The effect of date class was also highly significant ($P \leq 0.0001$), with plants from the middle date class producing significantly more leaf area than those from the late date class. All other effects were nonsignificant.

Cross type had a significant effect on both the number of ramets produced ($P = 0.0339$) and ramet mass per individual ($P = 0.0002$; Tables 2, 3)z. *Iris hexagona* (8.72 ± 0.69 ramets), IH × IF (7.20 ± 0.66 ramets) and IF × IH (6.50 ± 0.85 ramets), produced significantly more ramets than did *I. fulva* (5.62 ± 0.61 ramets). In terms of ramet mass, IH × IF (80.20 ± 7.01 g), *I. hexagona* (79.89 ± 7.34 g), and IF × IH (67.76 ± 8.99 g) were significantly larger than *I. fulva*

TABLE 3. Extended.

Weeks to first flower			Number of flowers			Number of seeds			Percent fruit set		
df	MS	P	df	MS	P	df	MS	P	df	MS	P
2	11.34	**0.0218**	2	4.65	**0.0421**	2	1181	0.6402	2	0.93	**0.0006**
1	0.16	0.8066	1	0.01	0.9534	1	182	0.7932	1	0.02	0.7025
1	0.06	0.8814	1	0.01	0.9281	1	2589	0.3260	1	0.07	0.4179
2	0.59	0.8050	2	1.11	0.4480	2	757	0.7508	2	0.07	0.5061
2	0.55	0.8168	2	1.35	0.3797	2	2269	0.4282	2	0.04	0.6697
1	1.26	0.4981	1	0.31	0.6351	1	95	0.8496	1	0.01	0.8125
1	0.41	0.6993	1	0.83	0.4401	1	81	0.8616	1	0.01	0.8166
43	2.71		43	1.36		43	2623		43	0.11	

(40.65 ± 6.47 g). Date class had a significant effect on both the number of ramets produced ($P = 0.0108$) and ramet mass per individual ($P = 0.0009$), with the middle date class producing a significantly greater number and mass of ramets than the late date class. Furthermore, the cross × date class interaction was significant for both measures ($P = 0.0007$ and $P = 0.0116$, respectively). In both cases, the significant interaction term is the result of a sharp decrease in the value of *I. hexagona* from the middle to the late date classes relative to the other three cross types.

Sexual Reproduction

The analyses of the flowering and seed production data were carried out in the absence of the *I. hexagona* individuals, as only one of the 76 *I. hexagona* individuals in the middle and late date classes flowered. The effect of cross type on percent flowering was significant ($P = 0.0188$; Tables 2, 3). A significantly higher percentage of *I. fulva* (64.24 ± 5.86%) individuals flowered when compared to the IF × IH cross type (23.73 ± 8.41%). IH × IF was not significantly different from either of the other two cross types (53.46 ± 9.01%). The effect of date class was also significant ($P = 0.0018$) with a higher proportion of individuals flowering in the middle date class than in the late date class. This effect was most likely a result of the fact that the individuals in the middle date class, having germinated earlier, were more mature during the flowering season than the individuals in the late date class. In addition, the cross × date interaction was significant ($P = 0.0068$). This interaction was caused by a decrease in percent flowering from the middle to late date classes in the *I. fulva* and IF × IH individuals, while percent flowering remained approximately constant from the middle to late date classes in IH × IF individuals.

The number of weeks to first flower differed significantly among cross types ($P = 0.0218$; Tables 2, 3). IH × IF individuals flowered significantly sooner after germination than did *I. fulva* individuals (26.36 ± 0.49 weeks vs. 28.23 ± 0.36 weeks, respectively). The number of weeks from germination to first flower for IF × IH individuals was intermediate to and not significantly different from the other two cross types (27.68 ± 0.55 weeks). All other effects were nonsignificant.

While the number of flowers produced per individual varied significantly among cross types ($P = 0.0421$), none of the model variables had a significant effect on seed production per indi-

vidual (Tables 2, 3). *Iris fulva* plants produced significantly more flowers (5.93 ± 0.26 flowers) than did IH × IF plants (4.94 ± 0.35 flowers), while IF × IH was not significantly different from the other two cross types (5.12 ± 0.39 flowers). In contrast, IF × IH plants produced the most seeds (114.95 ± 17.21 seeds), followed by IH × IF (113.68 ± 15.17 seeds) and *I. fulva* (99.95 ± 11.22 seeds). Finally, the effect of cross type on percent fruit set was highly significant ($P = 0.0006$; Tables 2, 3). IH × IF plants exhibited a significantly higher percent fruit set (88.70 ± 7.34%) than did *I. fulva* plants (41.11 ± 5.43%), whereas IF × IH (58.54 ± 8.32%) was intermediate to and not significantly different from the other two cross types. All other effects were nonsignificant.

DISCUSSION

On average, F_1 hybrids between *I. fulva* and *I. hexagona* performed as well as or better than their parental species for all fitness components measured (Table 2). In fact, even when the differences between cross types were nonsignificant, the hybrids outperformed at least one, and often both, of their parental species. There was, in fact, no evidence that the F_1 hybrids were less fit than their parents. Therefore, the results of this study are inconsistent with both the tension zone and mosaic models of hybrid zone evolution, which assume that hybrids are uniformly unfit relative to their parental taxa across all environments (Barton and Hewitt 1985; Harrison 1986; Howard 1986).

In this study, the F_1 hybrids were intermediate to, but not significantly different from, their parental species in terms of both ramet number and ramet mass. In view of the observed differences in vegetative growth (Fig. 1), however, it is clear that the IH × IF hybrids were more vigorous than both of their parents. In addition, both F_1 cross types were equivalent to *I. fulva* and superior to *I. hexagona* (which had extremely low levels of flowering) in flowering percentage and the number of flowers and seeds produced; the IH × IF cross type was superior to the other cross types in terms of the number of weeks from germination to first flower and percent fruit set. The low levels of sexual reproduction that we observed in *I. hexagona* were not completely unexpected, as *I. hexagona* tends to rely more heavily on clonal reproduction and less on sexual reproduction than does *I. fulva* (Arnold and Bennett 1993). In view of these apparently different life-history strategies, it is important to note that the

TABLE 4. Fitness estimates based on the rank performance of *I. fulva*, IF × IH, IH × IF, and *I. hexagona* in each of the 12 traits measured. Each cross type was assigned a performance score (1, 2, 3, or 4) based on its relative rank order in each trait (i.e., best = 4, worst = 1). Relative fitnesses for each life-history stage were then calculated as the mean of the scores within that stage divided by the mean score of the best cross type in that stage. Composite fitness estimates for each cross type are the mean of the fitness estimates from each of the four stages. Analysis of the fitness estimates from each of the four stages using a Kruskal-Wallis test revealed a nearly significant effect of cross type ($\chi^2 = 6.60$, df = 3, $P = 0.0857$).

Life-history stage	*I. fulva*	IF × IH	IH × IF	*I. hexagona*
Germination	1.00	0.57	1.00	0.29
Growth	0.33	0.67	1.00	0.50
Clonal reproduction	0.29	0.57	1.00	1.00
Sexual reproduction	0.88	0.94	1.00	0.31
Composite fitness	0.63	0.69	1.00	0.53

hybrids did not simply outperform each parent in its less utilized mode of reproduction. Rather, the hybrids appear to combine the clonal reproduction of *I. hexagona* with the relatively high levels of sexual reproduction characteristic of *I. fulva*. This conclusion is supported by the fitness estimates for each of the four life-history stages studied (Table 4). These estimates reveal that the IH × IF cross type performed as well as or better than both parental taxa in every stage. Furthermore, the composite fitness estimates are higher for the IH × IF and IF × IH cross types compared to their parents, and the variation among cross types is nearly significant ($P = 0.0857$). Overall, these findings are in close agreement with those of Graham et al. (1995) and Wang et al. (1997) in *Artemisia* and Emms and Arnold (1997) in *Iris*, all of whom found evidence for elevated hybrid fitness relative to their parents.

Graham et al. (1995) found that herbivore loads on hybrid individuals between *A. tridentata* ssp. *tridentata* and *A. tridentata* ssp. *vaseyana* were not significantly greater than on their progenitor subspecies. In addition, they found that hybrid individuals produced significantly more flowers than both of their progenitor subspecies, and that hybrid seeds germinated at a significantly higher rate than *A. t.* ssp. *vaseyana* seeds. Furthermore, in a series of reciprocal transplants, Wang et al. (1997) found that hybrids between these same two *Artemisia* subspecies were significantly more fit than their progenitors in the hybrid habitat. The authors of these studies concluded that the hybrids performed as well or better than their parents in some habitats.

In a study involving reciprocally transplanted *I. fulva*, *I. hexagona*, and F_1 and F_2 hybrids, Emms and Arnold (1997) found that the hybrids performed at least as well as both parental classes at all sites in terms of leaf production. In addition, both hybrid classes performed slightly better than their parents in ramet mass. Finally, there were no significant differences among cross types in survival or number of new ramets at any of the sites. These results led the authors to conclude that once their rhizomes are established, hybrids between *I. fulva* and *I. hexagona* can reproduce clonally at least as well as their parents in all habitats, and can outperform them in some habitats. The results of the current study,

which complement the findings of Emms and Arnold (1997), extend their work to include all stages of the life history of these species and their hybrids.

In view of the large number of cases of natural hybridization that have been documented in the Louisiana irises (e.g., Viosca 1935; Randolph et al. 1967), it appears that hybridization events have lasting effects on the genetic structure of populations. Although the establishment of F_1 hybrids between *I. fulva* and *I. hexagona* is an extremely rare event in natural populations (Arnold et al. 1990a, b, 1991, 1992; Nason et al. 1992; Arnold 1993; Cruzan and Arnold 1993), it must be kept in mind that these species are clonally reproducing perennials. Relative to species without the capacity for clonal reproduction, this should increase the likelihood of introgression over evolutionary time (Stebbins 1959; Ellstrand et al. 1996). It therefore seems likely that the rare establishment of these individuals will provide repeated opportunities for the production of advanced generation hybrids (i.e., backcrosses, F_2, F_3, etc.). Indeed, the introduction of F_1 hybrid individuals into a population of *I. fulva* and *I. hexagona* led to a significant increase in the overall rate of hybridization (Hodges et al. 1996).

The results of the present study indicate that F_1 hybrids between *I. fulva* and *I. hexagona* germinate, survive, and reproduce at least as well as, if not better than, their parental species in the greenhouse environment. In contrast to these findings, the tension zone and mosaic models assume that natural selection acts against all hybrids, regardless of environment. Our results, therefore, support the view that natural hybridization can be a creative evolutionary force (e.g., Anderson 1949; Anderson and Stebbins 1954; Lewontin and Birch 1966; Dowling and DeMarais 1993; B. R. Grant and Grant 1993; Arnold 1997), both in terms of the production of novel genotypes that are relatively more fit than their parents and in the formation of new taxa. This being said, there are two important caveats that must be kept in mind. First, because we have only estimated fitness over a period of 38 weeks, it is possible that our results do not reflect lifetime fitness. This study does, however, provide an analysis of fitness during the seed-to-seedling and seedling-to-adult transitions, as well as the first full flowering season. Not only is early fecundity an important determinant in lifetime fitness (Cole 1954), but the life-history stages analyzed also represent a period of time when selection might be expected to be strong (Cruzan and Arnold 1994). The second caveat results from our analysis being performed under greenhouse conditions. It is thus not possible to extrapolate the performance of these four cross types into their natural habitats based on our findings. Our results do, however, clearly contradict the assumption of environment-independent hybrid inferiority. Further, as discussed above, the results of Emms and Arnold (1997) suggest that F_1 hybrids between these species are at least as fit as their parents in all natural habitats studied, and more fit than their parents in some habitats.

Overall, our findings indicate that there is a need to reevaluate the models currently used in interpreting hybrid zone data. However, this reevaluation will need to include studies of the fitness of hybrids and their parents across habitats. Reciprocal transplantation of individuals across environments will allow a test of the effect of habitat differences on the fitness of specific

hybrid genotypes, relative to their parents, across a wide range of life-history stages. These studies will provide critical data on hybrid fitness in both plant and animal systems.

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Corresponding Editor: A. Sakai