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GENETIC INTERACTIONS AND NATURAL SELECTION IN LOUISIANA IRIS HYBRIDS

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Abstract.—Hybridization between divergent lineages has long been assumed to give rise to unfavorable interactions between the parental genomes. These deleterious genetic interactions are further assumed to result in the production of hybrid offspring with decreased levels of viability and/or fertility. To test this assumption, we investigated the role of both nuclear and cytonuclear epistatic interactions in determining the frequencies of F_2 genotypes produced in crosses between two species of Louisiana iris, *Iris fulva* and *I. brevicaulis*. Overall, these crosses revealed a significant deficit of intermediate hybrid genotypes accompanied by an excess of parental-like genotypes, suggesting that genetic interactions may promote postmating reproductive isolation between these species. However, analyses of single and multilocus segregation patterns revealed a variety of negative and positive interactions between the genomes of the parental taxa at the nuclear and cytonuclear levels. Taken together, these results indicate that the traditional view that interactions between divergent genomes are always deleterious is an oversimplification. Rather, it seems likely that crosses between divergent lineages can lead to the production of both fit and unfit hybrid genotypes.

Key words.—Cytonuclear interactions, epistasis, Louisiana irises, natural hybridization, natural selection, RAPDs, reproductive isolation.

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The fundamental role of epistasis in evolution was first championed by Wright (1931). According to this view, natural selection acts to retain favorably interacting gene combinations. Therefore, as a result of the highly integrated nature of the genome, evolution may lead to the production of what Dobzhansky (1970) has termed "coadapted" gene complexes. In contrast, Fisher (1930) argued that natural selection acts primarily on single genes rather than on gene complexes. In this case, natural selection favors alleles that elevate fitness, on average, across all possible genetic backgrounds within a lineage. In both Wright's and Fisher's scenarios, however, adaptive evolution is assumed to proceed independently within distinct evolutionary lineages. Therefore, crosses between divergent lineages may result in unfavorable gene combinations. As a result of these deleterious genetic interactions, hybrid offspring are generally expected to exhibit decreased levels of viability and/or fertility relative to their parental taxa (Mayr 1963).

The occurrence of hybrid breakdown, which manifests itself in the form of hybrid inviability or sterility, has long been taken as evidence of unfavorable interactions between the genomes of the parental taxa (e.g., Dobzhansky 1936, 1950, 1970; Muller and Pontecorvo 1940; Mayr 1963). This conclusion is based on the observation that hybrid breakdown often occurs in the F_2 and later generations and, therefore, may be a direct result of recombination between the genomes of the parental taxa (Dobzhansky 1950, 1970). In fact, a variety of studies have shown that the disruption of interactions within parental genomes can produce hybrid breakdown in both plant and animal systems (e.g., Dobzhansky 1936; Muller and Pontecorvo 1940; Coates and Shaw 1984; Burton 1990; Palopoli and Wu 1994; Li et al. 1997). As such, genetic interactions may promote postmating reproductive isolation between taxa.

In contrast to the observation of hybrid breakdown, several authors have reported the occurrence of favorable interactions between divergent parental genomes at both the nuclear and cytonuclear levels (e.g., Anderson and Stebbins 1954; Van Valen 1963; Lewontin and Birch 1966; MacRae and Anderson 1988; Hutter and Rand 1995; Rieseberg et al. 1996). This phenomenon has been documented at the interpopulational and interspecific levels in plants and animals and suggests that, in addition to promoting postmating reproductive isolation between lineages, recombination and segregation of the parental genomes may sometimes generate adaptive genetic variation. Therefore, crosses between genetically divergent lineages may provide the raw material necessary for adaptive evolution (Anderson 1949; Anderson and Stebbins 1954). Here we report the results of an experiment designed to study the effects of genetic interactions on the outcome of crosses between two species of Louisiana iris.

Iris fulva Ker-Gawler and *I. brevicaulis* Raf. (Iridaceae) are members of the Louisiana iris species complex. *Iris fulva* has brick red flowers, is predominantly hummingbird pollinated and is generally associated with the shady understory habitats typically found along the banks of bayous of the Mississippi River (Viosca 1935; Cruzan and Arnold 1993). *Iris brevicaulis* has blue flowers, is predominantly bumblebee pollinated and tends to occur in drier oak forest and pasture habitats (Viosca 1935; Cruzan and Arnold 1993). Although these species prefer quite different habitats, they occur sympatrically in southern Louisiana, and interspecific matings have led to the production of numerous hybrid populations throughout this region (Viosca 1935; Cruzan and Arnold 1993, 1994).

Analyses of natural Louisiana iris hybrid populations have revealed a relatively high frequency of parental and parental-like genotypes, accompanied by an extremely low frequency of intermediate hybrid genotypes (Arnold et al. 1990a,b, 1991, 1992; Nason et al. 1992; Arnold 1993; Cruzan and Arnold 1993, 1994). In one such population consisting of *I. fulva*, *I. brevicaulis*, and their hybrids, Cruzan and Arnold

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(1994) found that, although intermediate hybrid genotypes are rare or absent at the adult stage, they occur at a significantly higher frequency in seeds. Furthermore, an analysis of viability at the late seed stage revealed significant differences among the various hybrid and parental genotypes. In particular, there was a tendency for intermediate genotypic classes to have relatively low levels of seed viability when compared to the parental and parental-like genotypic classes. This result is consistent with the hypothesis that intermediate hybrid genotypes exhibit decreased viability relative to parental and parental-like hybrid genotypes as a result of incompatibilities between the *I. fulva* and *I. brevicaulis* genomes. In the present study, we investigated the role of both nuclear and cytonuclear epistatic interactions in determining the frequencies of F_2 genotypes produced in crosses between *I. fulva* and *I. brevicaulis*.

Materials and Methods

Plant Material

The greenhouse populations of *I. fulva* and *I. brevicaulis* used in this study were derived from rhizomes collected from natural populations of each species. The crossing scheme employed in this study was designed to produce F_2 seeds on alternate cytoplasmic backgrounds. Hand-pollinations using heterospecific pollen were performed on flowers of *I. fulva* and *I. brevicaulis* to produce reciprocal F_1 hybrids (progeny from these crosses are denoted $F_{1(F)}$ and $F_{1(B)}$, respectively). The resulting F_1 individuals were crossed to produce the F_2 generation by hand-pollinating $F_{1(F)}$ flowers with pollen from $F_{1(F)}$ individuals and $F_{1(B)}$ flowers with pollen from $F_{1(B)}$ individuals (progeny from these crosses are denoted $F_{2(F)}$ and $F_{2(B)}$, respectively). All F_1 crosses were made during the spring of 1993 and all F_2 crosses were made during the spring of 1995.

Seeds from both F_2 cross types were planted in 72 cell flats in a completely randomized design in the Botany Department greenhouse at the University of Georgia. Seedling emergence was recorded during weekly censuses from 15 May 1996 through 20 November 1996 (approximately six months), after which time seedling emergence ceased. Eight to 12 F_2 seedlings from each of 17 $F_{1(F)}$ and 17 $F_{1(B)}$ maternal individuals were randomly selected for inclusion in the genetic analysis (N = 197 $F_{2(F)}$ and 186 $F_{2(B)}$ seedlings). Leaf samples were collected from each selected individual, placed on ice, and returned to the lab for DNA extraction.

cpDNA and RAPD Analyses

Total genomic DNA was extracted from each of the 197 $F_{2(F)}$ and 186 $F_{2(B)}$ leaf samples using the methods of Edwards et al. (1991) for small tissue samples. All DNA samples were resuspended in 50 μL of water and stored at −20°C. Chloroplast DNA (cpDNA) inheritance has previously been shown to be almost strictly maternal in crosses between *I. fulva* and *I. hexagona* (Cruzan et al. 1993). To confirm this result for crosses between *I. fulva* and *I. brevicaulis*, we assayed a randomly selected subset of 53 $F_{2(F)}$ and 51 $F_{2(B)}$ individuals for their genotype at a species-specific cpDNA marker (Arnold et al. 1991; Arnold 1993; Cruzan and Arnold 1993).

All sampled individuals were genotyped for a series of five randomly amplified polymorphic DNA markers (RAPDs; Williams et al. 1990). The first three of these markers are dominant and diagnostic for *I. fulva* (F154A, F165A, F169B), the next is dominant and diagnostic for *I. brevicaulis* (B156A), and the last marker is codominant and fixed for alternate alleles in the two species (L180; Arnold 1993; Cruzan and Arnold 1993, 1994). Protocols for reaction mixtures, PCR conditions, and gel electrophoresis followed those of Arnold (1993).

Genetic Data Analyses

Observed single-locus segregation patterns for each F_2 cross type were compared to their expected values using χ^2 goodness-of-fit tests. Expected values were based on the predicted 3:1 segregation of dominant markers and 1:2:1 segregation of codominant markers in the F_2 generation. Because of the relatively large number of tests conducted, we used the sequential Bonferroni procedure of Holm (1979) to evaluate the statistical significance of each test.

Pairwise associations among the RAPD markers for each F_2 cross type were estimated using the frequencies of marker genotypes. For a pair of dominant loci A and B, the deviation from random association can be estimated as:

$$D_{AB} = p_{AB} - p_A p_B, \quad (1)$$

where p_{AB} is the frequency of individuals that exhibit the *I. fulva* genotype at both loci, p_A is the frequency of the *I. fulva* genotype at locus A, and p_B is the frequency of the *I. fulva* genotype at locus B (Cruzan and Arnold 1993). This calculation is analogous to the estimation of gametic disequilibrium between two loci, and the null hypothesis H_0: $D_{AB} = 0$ can be evaluated as a χ^2 test with one degree of freedom following Weir (1996). For the purpose of these calculations, L180 was treated as a dominant marker for *I. brevicaulis*, and significance levels were adjusted using the sequential Bonferroni procedure of Holm (1979).

Three-way associations among all possible combinations of the five RAPD markers for each F_2 cross type were estimated as:

$$D_{ABC} = p_{ABC} - p_A D_{BC} - p_B D_{AC} - p_C D_{AB} - p_A p_B p_C, \quad (2)$$

where p_{ABC} is the frequency of individuals that exhibit the *I. fulva* genotype at all three loci: p_A, p_B, and p_C are the frequency of the *I. fulva* genotype at locus A, B, and C, respectively, and the two-way disequilibria D_{BC}, D_{AC}, and D_{AB} are defined as above. This calculation is analogous to the estimation of gametic disequilibrium among three loci, and the null hypothesis H_0:$D_{ABC} = 0$ can be evaluated as a χ^2 test with one degree of freedom following Weir (1996). Again, for the purpose of these calculations, L180 was treated as a dominant marker for *I. brevicaulis* and the significance levels were adjusted using the sequential Bonferroni procedure of Holm (1979).

Genotypic distributions of the seedlings from each F_2 cross type were generated by assigning each seedling a hybrid index score (0 to 5) based on the number of *I. brevicaulis* RAPD markers they possessed. For the dominant RAPD markers, either the presence of an *I. brevicaulis* marker or the absence

TABLE 1. Single-locus genotypic distributions for all four dominant RAPD markers. At markers F154A, F165A, and F169B the "Present" genotype is dominant for *I. fulva* and the "Absent" genotype is recessive for *I. brevicaulis*. At B156A the "Present" genotype is dominant for *I. brevicaulis* and the "Absent" genotype is recessive for *I. fulva*. Expected values are based on the predicted 3:1 segregation of a dominant marker in the F_2 generation. χ^2 values are for goodness-of-fit tests with one degree of freedom.

		Present		Absent		
Locus	Cross	Observed	Expected	Observed	Expected	χ^2
F154A	$F_{2(F)}$	148	147.75	49	49.25	0.0017
F154A	$F_{2(B)}$	137	139.50	49	46.50	0.1792
F165A	$F_{2(F)}$	151	147.75	46	49.25	0.2860
F165A	$F_{2(B)}$	146	139.50	40	46.50	1.2115
F169B	$F_{2(F)}$	147	147.75	50	49.25	0.0152
F169B	$F_{2(B)}$	142	139.50	44	46.50	0.1792
B156A	$F_{2(F)}$	126	147.75	71	49.25	12.8061**
B156A	$F_{2(B)}$	132	139.50	54	46.50	1.6129

** $P < 0.01$, adjusted using the sequential Bonferroni procedure of Holm (1979).

of an *I. fulva* marker was interpreted as an *I. brevicaulis* genotype. For the codominant RAPD marker (L180), only the allele diagnostic for *I. brevicaulis* was counted. The resulting distributions were compared to the distribution expected under simple Mendelian segregation of the five RAPD loci using χ^2 goodness-of-fit tests.

RESULTS

Similar to what has previously been shown in crosses between *I. fulva* and *I. hexagona* (Cruzan et al. 1993), cpDNA inheritance is almost strictly maternal in crosses between *I. fulva* and *I. brevicaulis*. All 53 $F_{2(F)}$ individuals that were genotyped carried the cpDNA haplotype characteristic of *I. fulva*, whereas 50 of 51 $F_{2(B)}$ individuals carried the cpDNA haplotype characteristic of *I. brevicaulis*. The remaining $F_{2(B)}$ individual, which carried the cpDNA haplotype of *I. fulva*, resulted from rare paternal leakage. The observed rate of paternal leakage was, therefore, about 1%. In view of these findings, cpDNA haplotypes were inferred for the remainder of the individuals based on their cross type. All $F_{2(F)}$ individuals were assumed to have the *I. fulva* cpDNA and all $F_{2(B)}$ individuals were assumed to have the *I. brevicaulis* cpDNA.

Single-Locus Disequilibria

Observed single locus genotypic distributions for all three dominant *I. fulva* diagnostic markers (F154A, F165A, and F169B) were not significantly different from their expected distribution in either cross type (all $P > 0.25$; Table 1). In contrast, the observed distribution of genotypes at B156A was not significantly different from the expected 3:1 distribution in the $F_{2(B)}$ cross type ($P = 0.20$), but deviated significantly from the expected distribution in the $F_{2(F)}$ cross type ($P < 0.01$; Table 1). In other words, B156A exhibited an excess of *I. fulva* genotypes and a deficit of *I. brevicaulis* genotypes on the *I. fulva* cpDNA background, whereas there were no significant deviations from expectation on the *I. brevicaulis* cpDNA background.

The observed genotypic distribution at the codominant marker (L180) deviated significantly from the expected 1:2:1 distribution on both cpDNA backgrounds (both $P < 0.001$; Table 2). The main cause of this deviation was a complete absence of the *I. brevicaulis* homozygote (B/B) in both F_2 cross types. This result suggests that the B/B genotype is lethal in an F_2 recombinant nuclear background. Assuming this to be true, we would expect to see a 2:1 ratio of B/F to F/F genotypes. In fact, this is very close to what we observed. On the *I. brevicaulis* cpDNA background, the observed distribution of B/F and F/F genotypes is not significantly different from the 2:1 expectation ($\chi^2 = 0.39$, df = 1, $P = 0.53$), whereas on the *I. fulva* background there is a slight but statistically significant heterozygote (B/F) excess ($\chi^2 = 4.27$, df = 1, $P < 0.05$) relative to the expected 2:1 distribution.

Multilocus Disequilibria

The pairwise disequilibria statistics for all possible pairs of the five RAPD loci in both F_2 cross types are listed in Table 3. There is a highly significant ($P < 0.001$) positive association between L180 and B156A on both cpDNA backgrounds, indicating a positive association between intraspecific alleles at the two loci. In addition, there is a significant positive association ($P < 0.01$) between F165A and F169B on the *I. brevicaulis* cpDNA background, but not on the *I. fulva* background. This result indicates that, although genotypes at F165A and F169B are randomly associated on the *I. fulva* background, there is a positive association between intraspecific alleles at the two loci on the *I. brevicaulis* background. No other pairs of loci exhibit significant nonrandom associations on either cpDNA background. In contrast to the two-locus results, there is little evidence of nonrandom three-way associations. None of the three-way associations on either of the two cpDNA backgrounds are statistically significant, although F154A, B156A, and L180 exhibit a significantly positive three-way association ($P < 0.05$) on the *I. fulva* background prior to application of the sequential Bonferroni adjustment (data not shown). Although this suggests that there is a positive association among intraspecific alleles

TABLE 2. Single-locus genotypic distributions for the codominant RAPD marker. Expected values are based on the predicted 1:2:1 segregation of a codominant marker in the F_2 generation. χ^2 values are for goodness-of-fit tests with two degrees of freedom.

		B/B		B/F		F/F		
Locus	Cross	Observed	Expected	Observed	Expected	Observed	Expected	χ^2
L180	$F_{2(F)}$	0	49.25	145	98.50	52	49.25	71.3553***
L180	$F_{2(B)}$	0	46.50	128	93.00	58	46.50	62.5161***

*** $P < 0.001$, adjusted using the sequential Bonferroni procedure of Holm (1979).

TABLE 3. Disequilibrium estimates (D_{AB}) for all pairs of RAPD markers on both cytoplasmic backgrounds. Values greater than zero indicate positive intraspecific associations, whereas values less than zero indicate negative intraspecific associations. The statistical significance of each estimate was evaluated following the methods of Weir (1996). Values above the diagonal refer to associations on the *Iris fulva* cytoplasmic background, and those below the diagonal refer to associations on the *I. brevicaulis* cytoplasmic background.

	F154A	F165A	F169B	B156A	L180
F154A	—	−0.0022	0.0130	−0.0119	−0.0156
F165A	−0.0136	—	0.0118	−0.0021	0.0342
F169B	0.0183	0.0298**	—	−0.0151	−0.0142
B156A	−0.0042	−0.0075	0.0134	—	0.0927***
L180	0.0069	0.0133	0.0092	0.0654***	—

** $P < 0.01$, *** $P < 0.001$, adjusted using the sequential Bonferroni procedure of Holm (1979).

at these three loci on the *I. fulva* background, this may be a spurious result.

Genotypic Distributions

The observed and expected genotypic distributions for both F_2 cross types as well as the pooled data are depicted in Figure 1. Because categories with small expected cell sizes can contribute disproportionately to the χ^2 test statistic, we pooled classes 4 and 5 for all goodness-of-fit tests. The distribution of $F_{2(F)}$ seedlings is significantly different from the expected distribution ($\chi^2 = 14.64$, df = 4, $P < 0.01$). This significant deviation mainly results from an excess of parental-like (class 0) hybrid individuals accompanied by a deficit of intermediate (class 3) hybrid genotypes (Fig. 1A). Similarly, the $F_{2(B)}$ seedling distribution is significantly different from the expected distribution ($\chi^2 = 19.60$, df = 4, $P < 0.001$) due to an excess of parental-like (classes 0, 1, and 4/5) genotypes, and a deficit of intermediate (classes 2 and 3) genotypes (Fig. 1B). Because a χ^2 test for independence indicated that the two genotypic distributions are not significantly different from each other ($\chi^2 = 1.97$, df = 1, $P = 0.32$), we pooled the data and repeated the analyses. Not surprisingly, the pooled distribution differs significantly from expected ($\chi^2 = 31.75$, df = 4, $P < 0.001$), once again owing to a deficit of intermediate genotypes (classes 2 and 3) and an excess of parental-like genotypes (classes 0, 1, and 4/5; Fig. 1C).

DISCUSSION

The observed distribution of genotypes on both cpDNA backgrounds suggests that selection acts against intermediate hybrid genotypes, thereby producing an excess of parental-like genotypes (Fig. 1). Although this pattern is consistent with the observed deficit of intermediate hybrid genotypes in natural Louisiana iris populations, an important difference must be pointed out. Previous analyses of natural hybrid populations have documented not only a deficit, but a complete absence of intermediate genotypes among adult individuals (Arnold et al. 1990a,b, 1991, 1992; Nason et al. 1992; Arnold 1993; Cruzan and Arnold 1993, 1994). These genotypes are present at the late seed stage, suggesting that selection acting between the seed and adult stages is responsible for the observed absence of intermediate genotypes. Indeed, Cruzan and Arnold (1994) documented relatively low levels of seed viability in intermediate genotypic classes in a natural population consisting of *I. fulva, I. brevicaulis*, and their hybrids. Although the results of the present study can, in part, account for the lack of intermediate genotypes in the wild, the apparent seed-to-seedling selection that we have documented appears to be only part of the story. Rather, it seems likely that additional, perhaps environmentally mediated selection pressures during the seed-to-seedling and/or seedling-to-adult transitions contribute to the observed genotypic distributions in natural populations.

On the basis of the single-locus segregation patterns (Table 1), it appears that all three *I. fulva* diagnostic markers (F154A, F165A, and F169B) are selectively neutral on both cytoplasmic backgrounds (but see discussion of multilocus disequilibria below). In contrast, the segregation pattern observed at B156A is indicative of cytonuclear selection. Although the observed segregation pattern on the *I. brevicaulis* cytoplasmic background does not differ significantly from expected, there is a significant excess of *I. fulva* genotypes and a deficit of *I. brevicaulis* genotypes on the *I. fulva* background. This sort of nonreciprocal deviation from expected frequencies can best be explained in terms of cytonuclear interactions. Hybridization appears to have disrupted favorable cytonuclear interactions in *I. fulva*. This disruption produced selection against the *I. brevicaulis* genotype at B156A on the *I. fulva* cytoplasmic background. Because the cpDNA serves only as a marker for the maternally inherited cytoplasm, it is impossible to attribute these effects to interactions of the nuclear genome with the chloroplast genome itself. It does seem reasonable, however, to conclude that *I. fulva* alleles at one or more loci linked to B156A interact favorably with some component of the maternally inherited *I. fulva* cytoplasm (e.g., the chloroplast or mitochondrial genomes or a cytoplasmic parasite). Similar phenomena have been documented in a variety of systems including *Epilobium* (reviewed in Michaelis 1954), *Culex* (Laven 1956), *Tribolium* (Wade and Stevens 1985), *Drosophila* (e.g., Hoffman et al. 1986; Clark and Lyckegaard 1988), and *Nasonia* (Breeuwer and Werren 1995). These results suggest that cytonuclear interactions may influence the directionality of introgression or, alternatively, promote reproductive isolation between a wide variety of taxa.

The complete absence of the *I. brevicaulis* (B/B) genotype at L180 indicates that this marker is tightly linked to a locus involved in epistatic lethal selection (Table 2). Although the B allele has no apparent deleterious viability effects in pure *I. brevicaulis* individuals, it appears to behave as a recessive lethal in the recombinant F_2 nuclear background. Because the B allele seems to be harmless in pure *I. brevicaulis* individ-

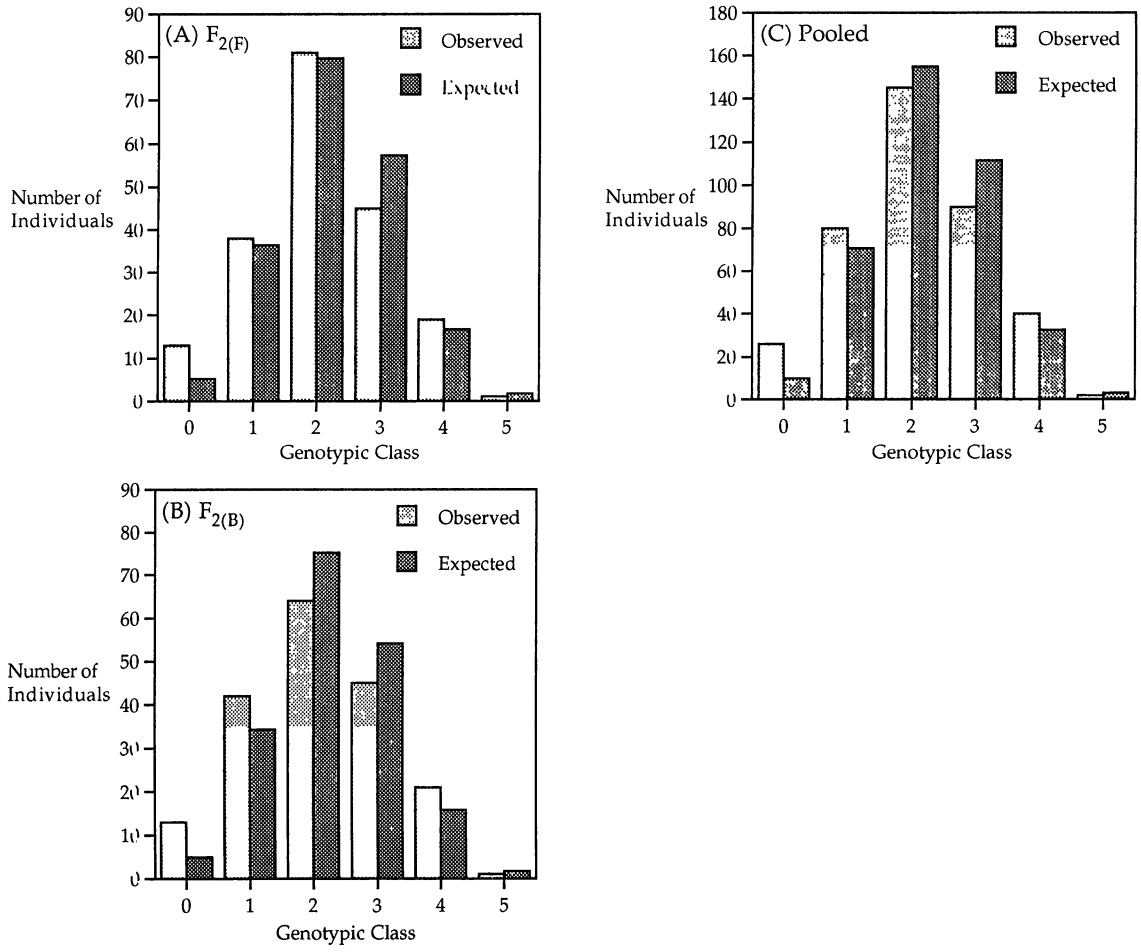

FIG. 1. Observed and expected genotypic distributions of F_2 seedlings on (A) the *Iris fulva* cpDNA background ($F_{2(F)}$); (B) the *I. brevicaulis* cpDNA background ($F_{2(B)}$); and (C) both cpDNA backgrounds (Pooled). All three observed distributions differ significantly from their expected distributions ($\chi^2 = 14.64$, df = 4, $P < 0.01$; $\chi^2 = 19.60$, df = 4, $P < 0.001$; $\chi^2 = 31.75$, df = 4, $P < 0.001$, respectively).

uals, its effect on viability must be a multilocus phenomenon. Apparently the disruption of favorable interactions within the *I. brevicaulis* nuclear genome leads to inviability of B/B homozygotes. Overall, this result is remarkably similar to what Dobzhansky (1946) has termed "synthetic" lethality, which refers to an epistatic fitness effect in which the interaction of otherwise harmless loci produces inviability. While there are numerous examples of synthetic lethals in *Drosophila*, the vast majority of these studies have dealt with intraspecific effects (reviewed in Thompson 1986). However, several studies have documented synthetic lethals at the interspecific level (e.g., Hollingshead 1930; Gerstel 1954; Watanabe 1979). Although these studies do not rule out the possibility that postmating reproductive isolation is mediated by genetic interactions at a large number of loci, they do suggest that one or a few loci of major effect can contribute substantially to hybrid breakdown.

A comparison of the observed segregation pattern at L180 to the 2:1 ratio expected under the assumption of B/B lethality revealed a significant heterozygote (B/F) excess on the *I. fulva*, but not *I. brevicaulis*, cytoplasmic background. Again, as in the case of B156A, the nonreciprocal nature of this deviation can best be explained in terms of cytonuclear interaction. In this case, it appears that the observed heterozygote excess results from selection favoring the B/F genotype on the *I. fulva*, but not *I. brevicaulis* cytoplasmic background. It is possible that the observed pattern could result from either overdominance (i.e., heterozygote advantage) or pseudo-overdominance (i.e., the antagonistic effects of two closely linked dominant loci in repulsion; Crow 1952) on the *I. fulva* background. In either case, this result clearly indicates that recombinant genotypes can be favored over the parental types.

The significant positive association between B156A and L180 on both cytoplasmic backgrounds suggests that conspecific alleles at these loci interact favorably (Table 3). Because we do not know the chromosomal location of these markers, it is possible that the observed association is a result of physical linkage. However, this explanation is untenable for two reasons. First, if B156A and L180 are indeed physically linked, we should see an excess of both coupling types. Rather, the significant positive association results from an excess of *I. fulva/I. fulva* two-locus genotypes accompanied by the expected number of *I. brevicaulis/I. brevicaulis* two-

locus genotypes and a deficit of both recombinant genotypes. Second, if the two loci are linked, epistatic lethal selection against the B/B genotype at L180 should produce a dramatic deficit of the *I. brevicaulis* genotype at B156A on both cytoplasmic backgrounds. Although we observed a significant deficit of the *I. brevicaulis* genotype on the *I. fulva* background, the observed segregation pattern on the *I. brevicaulis* background was not significantly different from expected. It therefore appears that the positive association between these loci is, in fact, due to favorable epistatic interactions between *I. fulva* alleles at these two loci rather than physical linkage.

In contrast to the single locus results (see above), the occurrence of significant positive disequilibrium between F165A and F169B indicates that these two loci do not behave neutrally (Table 3). Physical linkage cannot explain this association, as it occurs only on the *I. brevicaulis* cytoplasmic background. Rather, these results indicate the occurrence of favorable epistatic interactions between conspecific alleles (and unfavorable heterospecific interactions) at these loci. In addition, the fact that this association only occurs on one cytoplasmic background implies that F165A and F169B interact not only with each other, but with some component of the cytoplasm as well. The observed association results from an excess of both coupling types, indicating that the *I. fulva*/*I. fulva* two-locus genotype is favored over the recombinant genotypes on the heterospecific (i.e., *I. brevicaulis*) cytoplasmic background, but not on its own.

Taken together, the results of this study confirm that epistatic interactions play a major role in determining hybrid viability in crosses between *I. fulva* and *I. brevicaulis*. In contrast to the expectation that the genomes of divergent taxa will always interact unfavorably (Mayr 1963), we found evidence of favorable heterospecific interactions. Therefore, although many (if not most) interactions between divergent genomes may prove to be deleterious, it is possible that recombination and segregation of the parental genomes will yield favorable heterospecific interactions. This being said, there are several caveats that must be mentioned. First, there is no way to know the genotype of the ungerminated fraction of seeds. Although the majority of seeds from both cross types did, in fact, germinate (73.9% germination and 69.3% germination for the $F_{2(F)}$ and $F_{2(B)}$ cross types, respectively), it is possible that genotype-specific differences in seed dormancy may have contributed somewhat to the observed genotypic patterns. Second, it is possible that prezygotic factors such as meiotic drive or pollen competition among different F_1 pollen genotypes may have played a role in producing the observed genotypic patterns. As mentioned above, however, the deficit of intermediate genotypes in natural Louisiana iris hybrid populations is more apparent at the adult stage than at the seed stage (Cruzan and Arnold 1994). Coupled with the relatively low viability of seeds in intermediate genotypic classes, these results suggest that postzygotic selection against intermediate genotypes, rather than prezygotic factors, is responsible for the observed deficit of intermediate genotypes. Finally, it is important to note that the design of this experiment did not allow the detection of genes with relatively minor effects. Clearly, larger sample sizes and more markers would facilitate the detection of more subtle effects. Whether genes of relatively small effect exist, however, it appears that individual loci may contribute disproportionately to hybrid breakdown.

Overall, our findings indicate that the traditional view of uniformly deleterious interactions between divergent genomes is an oversimplification. Rather, it seems likely that these sorts of genetic interactions can lead to the production of hybrid genotypes with a wide range of fitnesses. Further, it has been suggested that positive epistatic interactions between divergent genomes may provide a basis for adaptive evolution (Anderson and Stebbins 1954; Lewontin and Birch 1966). Rieseberg et al. (1996) reached a similar conclusion in a study involving crosses between two species of sunflower, *Helianthus annuus* and *H. petiolaris*. In this case, the authors found that "a small percentage of alien genes do appear to interact favorably in hybrids," a result that led them to conclude that favorable interactions such as these may play a role in hybrid speciation. For this reason, genetic analyses such as the present one will continue to provide important data on both the nature of postmating reproductive barriers as well as the genetic basis of adaptation in hybrid lineages.

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