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Genetic Mapping of Species Boundaries in Louisiana Irises Using *IRRE* Retrotransposon Display Markers

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ABSTRACT

Genetic mapping studies provide insight into the pattern and extent of genetic incompatibilities affecting hybridization between closely related species. Genetic maps of two species of Louisiana Irises, *Iris fulva* and *I. brevicaulis*, were constructed from transposon-based molecular markers segregating in reciprocal backcross (BC_1) interspecific hybrids and used to investigate genomic patterns of species barriers inhibiting introgression. Linkage mapping analyses indicated very little genetic incompatibility between *I. fulva* and *I. brevicaulis* in the form of map regions exhibiting transmission ratio distortion, and this was confirmed using a Bayesian multipoint mapping analysis. These results demonstrate the utility of transposon-based marker systems for genetic mapping studies of wild plant species and indicate that the genomes of *I. fulva* and *I. brevicaulis* are highly permeable to gene flow and introgression from one another via backcrossing.

NATURAL hybridization is the interbreeding of genetically differentiated populations or species in the wild (Arnold 1997 as adapted from Harrison 1990), and it has been increasingly recognized as an important force in evolution (Arnold 1997; Rieseberg and Carney 1998; Rieseberg *et al.* 2003; Seehausen 2004). Hybridization contributes to genetic variation and evolution in numerous ways. It can serve as an avenue for the transfer of additive genetic variation that may contribute to adaptation, a process known as adaptive introgression (Anderson 1949; Arnold 1997; Grant and Grant 2002). Hybrid lineages may also form stable species that are distinct and reproductively isolated from the original parent taxa (Rieseberg *et al.* 2003). If hybrid progeny suffer from reduced fitness, selection may lead to reinforcement of reproductive isolation (Dobzhansky 1940; Blair 1955; Howard 1993; Marshall *et al.* 2002). Alternatively, hybridization may lead to the loss of genetic and phenotypic differentiation as one species is assimilated into another (Rhymer and Simberloff 1996; Huxel 1999; Anttila *et al.* 2000).

The evolutionary outcome of hybridization is in part determined by the nature and extent of species barriers, particularly genetic interactions that may occur between divergent genomes. Hybridization combines alleles that did not evolve in concert. A well-developed body of theory (Bateson 1909; Dobzhansky 1936, 1937; Muller 1940; Orr 1995; Turelli and Orr 2000; Orr and Turelli 2001) and experimental work (Fishman and Willis 2001; Harushima *et al.* 2001, 2002; Presgraves 2003; Presgraves *et al.* 2003; Barbash *et al.* 2004) has shown how this can result in genetic incompatibilities due to negative epistatic interactions between divergent alleles at different loci, resulting in hybrid genotypes with reduced fitness. Alleles or chromosomal regions contributing to reduced hybrid fitness will be less likely to introgress across species boundaries than those with neutral or positive fitness effects (Key 1968; Barton and Hewitt 1985; Navarro and Barton 2003). This means that reproductive isolation varies across the genome and may be very different from chromosome to chromosome, locus to locus, or even allele to allele (Key 1968; Barton and Hewitt 1985; Harrison 1990; Buerkle and Rieseberg 2001). As a result, the pattern and extent of genetic interactions that occur in hybrid genotypes sets the stage for whatever evolutionary outcomes ensue.

The Louisiana Irises (Iridaceae, section Apogon, series Hexagonae) have been the focus of numerous studies of interspecific hybridization and adaptive introgression (Small and Alexander 1931; Viosca 1935; Anderson 1948; Arnold *et al.* 2001; Johnston *et al.* 2003). These are long-lived, self-compatible, rhizomatous, perennial herbs that reproduce both sexually and clonally. Two members of this clade, *Iris fulva* and *I. brevicaulis*, are broadly sympatric throughout the Mississippi River valley of central North America, but are found in different habitats (Viosca 1935; Cruzan and Arnold 1993; Johnston *et al.* 2001b). *I. fulva* is found

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in intermittently flooded, forested wetlands throughout this range, while *I. brevicaulis* is normally found in drier, riparian-associated hardwood forests. *I. brevicaulis* is primarily bumble bee pollinated and possesses a suite of classic bee-pollination-associated floral traits: blue flowers marked with prominent white and yellow nectar guides, stiff upright tepals, and strongly scented flowers (Viosca 1935; Wesselingh and Arnold 2000). *I. fulva* is primarily hummingbird pollinated and possesses a markedly different floral phenotype: flowers of this species are solid red with protruding anthers and highly reflexed tepals (Viosca 1935; Wesselingh and Arnold 2000). *I. fulva* and *I. brevicaulis* also differ in flowering time: *I. fulva* populations begin flowering about a month earlier than *I. brevicaulis* populations in southern Louisiana (Cruzan and Arnold 1994).

Despite these differences in habitat, floral morphology, and phenology, these species form numerous hybrid zones in southern Louisiana, often as a consequence of habitat disturbance (Viosca 1935; Anderson 1948). Previous studies have detected extensive introgression of genetic markers in natural hybrid populations (Arnold *et al.* 1992; Cruzan and Arnold 1993; Johnston *et al.* 2001b). Still, these species remain morphologically distinct: genetic analyses of hybrid zones have indicated that the species mate assortively and that embryos with intermediate hybrid genotypes are more likely to be aborted (Cruzan and Arnold 1994). Consistent with a pattern of genetic incompatibilities in some hybrid genotypes is the occurrence of wide variation in pollen fertility of wild hybrid plants (Randolph *et al.* 1967). In addition, multilocus genetic incompatibilities have been detected in experimental F_2 hybrids (Burke *et al.* 1998). Initial formation of F_1 hybrids between *I. fulva* and *I. brevicaulis* appears to be extremely rare, and hybrid zone populations typically consist of genotypes resembling advanced generation backcross hybrids. This is in spite of the fact that F_1 hybrids of *I. fulva* and *I. brevicaulis* are vigorous and highly fertile (Johnston *et al.* 2003) and undergo normal meiosis. Pollinator studies have shown that gene flow via pollinator movements between F_1's and parental species types is likely to be high (Wesselingh and Arnold 2000).

In summary, previous results have detected genetic incompatibilities between *I. fulva* and *I. brevicaulis*, but the widespread occurrence of advanced generation hybrid plants in wild populations indicates that some genomic regions of these species must be permeable to introgression. This article presents a study assessing genetic interactions affecting hybridization between *I. fulva* and *I. brevicaulis* in reciprocal backcross 1 (BC_1) hybrids, which are a realistic model for hybrid genotypes likely to form in natural hybrid populations.

Studies of genetic incompatibilities between crop species and their relatives have taken advantage of well-saturated genetic maps available in agriculturally or economically important plants. These studies have provided a detailed picture of the genomic pattern of species barriers that limit introgression among cultivated subspecies [*e.g.*, rice (Harushima *et al.* 2001, 2002) and cotton (Jiang *et al.* 2000)], crops and their wild relatives [*e.g.*, tomato (Bernacchi and Tanksley 1997; Moyle and Graham 2005) and corn (Doebley *et al.* 1995)], and congeners with economically desirable traits [*e.g.*, eucalyptus (Myburg *et al.* 2004)]. In contrast, there are few studies of introgression in wild plant species at the level of detail provided by a genetic mapping approach. A number of these studies have focused on QTL mapping of the genetic basis of prezygotic barriers to gene flow in Mimulus (*e.g.*, Bradshaw *et al.* 1995, 1998; Lin and Ritland 1997; Fishman *et al.* 2002). Genetic mapping studies also provide a means of assessing the number and distribution of genetic factors that affect hybridization, including the identification of genomic regions permeable or resistant to introgression, or that directly contribute to breakdown in fitness components [*e.g.*, between species of Helianthus (Rieseberg *et al.* 1995, 1999; Kim and Rieseberg 1999; Buerkle and Rieseberg 2001)].

Genetic mapping studies require large numbers of molecular markers. Louisiana irises have a large physical genome with a haploid genome size of ~10,000 Mb (Kentner *et al.* 2003). In species with large genomes, the predominance of repetitive DNA may hamper the use of standard amplified fragment length polymorphisms (AFLPs) or RFLP- and microsatellite-based markers (Kinlaw and Neale 1997; Pfeiffer *et al.* 1997; Remington *et al.* 1999). Transposable element markers based on *IRRE* retrotransposons were developed for use in Iris by Kentner *et al.* (2003). The *IRRE* long terminal repeat (LTR) retrotransposons are a group of *TY3/gypsy*-like elements that compose 6–10% of the Louisiana Iris genome (Kentner *et al.* 2003). A modification of the amplified fragment length polymorphisms (Vos *et al.* 1995) procedure called transposon display is used to generate PCR products that are anchored in a transposable element and a flanking restriction site (Waugh *et al.* 1997; Ellis *et al.* 1998; Van den Broeck *et al.* 1998; Casa *et al.* 2000). The utility of LTR retrotransposon display markers has been demonstrated previously by mapping studies of, for example, barley (Waugh *et al.* 1997; Manninen *et al.* 2000) and pea (Ellis *et al.* 1998; Flavell *et al.* 1998; Kalendar *et al.* 1999). This article presents genetic maps of *I. fulva* and *I. brevicaulis* constructed from *IRRE* retrotransposon markers segregating in interspecific BC_1 hybrids. To identify genomic regions in which introgression of heterospecific alleles may be reduced or promoted, patterns of transmission ratio distortion (TRD) at marker loci were examined. The transmission ratios of parental alleles at marker loci were compared to expected Mendelian segregation and analyzed using the Bayesian approach of Vogl and Xu (2000). Mapping and segregation analyses indicate that

the majority of the genomes of both *I. fulva* and *I. brevicaulis* appear highly permeable to introgression from one another via backcrossing.

MATERIALS AND METHODS

Generation of BC_1 mapping populations: One wild-collected individual from each of the two species was used to make reciprocal interspecific backcross 1 (BC_1) mapping populations. In these crosses, the same individuals were used as both the F_1 parents and the recurrent parents for backcrossing. This was done to minimize the within-species genetic variation segregating in the experiment. The individuals used were collected from natural populations in southern Louisiana that had apparently not experienced hybridization with the other species, as determined by population genetic analysis or by population observations. The *I. fulva* individual, If174, was collected from a population in Terrebonne Parish, and the *I. brevicaulis* individual, Ib72, was collected from a population in St. Martin Parish. Flowers of Ib72 were crossed with pollen from If174 to make F_1 hybrids in 1997. Two F_1 hybrids, designated $F_1(2)$ and $F_1(3)$, were used to make backcross hybrids during the winter of 1999. Pollen from $F_1(3)$ was crossed onto flowers of several ramets (clones) of Ib72 to make *I. brevicaulis* BC_1 hybrids ($IbBC_1$), and pollen from $F_1(2)$ was crossed onto flowers of several ramets of If174 to make *I. fulva* BC_1 hybrids ($IfBC_1$). All flowers were emasculated prior to opening, and pollen was applied 2 days later after the stigmatic surface became receptive. All plants were housed in the University of Georgia Department of Plant Biology greenhouses.

Seeds were planted in flats during the following summer and monitored for germination for ~12 months. Seedlings were transplanted into 6-in. azalea pots shortly after germination. During September of 2000, a single rhizome of each BC_1 genotype was transplanted in an 8-in. azalea pot. All BC_1 plants as well as replicates of each parental genotype were randomly assigned positions in a single greenhouse. Plants were repotted annually by cutting out a single rhizome of each genotype and transplanting it to a new pot. Plants were randomly reassigned new positions each year at repotting. Mapping populations consisted of 230 $IbBC_1$ and ~225 $IfBC_1$ hybrid plants. All of the $IbBC_1$ plants and a subset of 120 of the $IfBC_1$ plants were used in the present study.

Genetic mapping: *DNA isolation:* Leaf tissue samples were collected from plants during the fall of 200l at which point plants had been cultivated in the greenhouse for ~18 months. Tissue samples were immediately snap frozen in liquid nitrogen. DNA was isolated using a modified CTAB protocol (Doyle and Doyle 1987; Soltis *et al.* 1991). DNA samples were treated with RNase A and standardized by comparison with λDNA of known concentration visualized on a 1% agarose gel with ethidium bromide.

Generation of transposon display genetic markers: Genetic markers were generated using the *IRRE* transposon display marker protocol of Kentner *et al.* (2003). This marker system employs a modified AFLP protocol to assay polymorphism in DNA sequence flanking insertion sites of *IRRE* LTR retrotransposons.

Genomic DNA (~500 ng) was digested with 50 units of *Eco*RI (New England Biolabs, Beverly, MA) at 37° for ~12 hr. Standard *Eco*RI AFLP adapters (Vos *et al.* 1995) were ligated to the digested DNA for ~12 hr at room temperature using 5 units of T4 DNA ligase and the manufacturer-supplied buffer (Invitrogen, San Diego).

Two rounds of PCR were performed: the first (preamplification) used a primer complementary to the *Eco*RI adapter sequence plus two selective bases, along with a primer specific to the 5′-end of the LTR of the *IRRE1-A1* (CCA AAC CAA ACC AAG CCA CAC TAA ACC) or *IRRE1-C* (ACA GGA ACA CTT TCC AAT TAC GT) retrotransposon (Kentner *et al.* 2003). Preamplification PCR was performed in 30-μl reaction volumes using 1 μl of restriction/ligation reaction product, 10 pmol of each primer, 1.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), 0.2 μM of each dNTP, 3.0 μM $MgCl_2$, and the manufacturer's buffer. PCR conditions were as follows: 72° for 2 min, 94° for 3 min, 35 cycles of 94° for 30 sec, 56° (*IRRE1-A1*) or 57° (*IRRE1-C*) for 30 sec, and 72° for 1 min, with a final elongation step at 72° for 3 min.

The second (selective) round of PCR amplification used *Eco*RI adapter primers plus four selective bases and *IRRE1-A1* or *IRRE1-C* LTR-specific primers nested within those used in the preamplification reactions (Kentner *et al.* 2003). Selective PCR reactions were performed in 10-μl volumes using 1.5 μl of 10:1 diluted preamplification product as template, 5 pmol of *Eco*RI adapter, 3 pmol ^{33}P-labeled *IRRE1-A1* (CGT ATA AAA TAC GTA CAC AAG AG) or *IRRE1-C* (TCC AAT TAC GTA TAA AAT ACG) primer, 1.5 units of AmpliTaq DNA polymerase (Applied Biosystems), 0.2 μM of each dNTP, 2.5 μM $MgCl_2$, and the buffer supplied with the enzyme. The cycling conditions were as follows: 94° for 3 min, 35 cycles of 94° for 30 sec, 56° for 50 sec, and 72° for 1 min, with a final elongation step at 72° for 4 min. PCR products were resolved on a 6% polyacrylamide sequencing gel and visualized by autoradiography. Bands were scored by eye for genetic analysis.

Estimation of heterozygosity at IRRE transposon display loci: Heterozygosity (H_e) of the Ib72 and If174 parents was assessed directly by scoring segregation of all alleles in recurrent backcross progeny. In other words, heterozygosity of the Ib72 parent allele markers was estimated by observing the segregation of these alleles in the $IbBC_1$'s, for which Ib72 served as the recurrent parent. Likewise, the heterozygosity of If174 was determined by observing the segregation of If174 alleles in $IfBC_1$ hybrids. This method does underestimate the heterozygosity of the recurrent parent because bands heterozygous in the recurrent parent but homozygous present in the F_1 parent will be scored as homozygous present in the BC_1 progeny. All bands that were clearly present in the parent lanes were scored to estimate heterozygosity whether or not they were subsequently used in linkage mapping and whether or not they were species specific (Figure 1). Heterozygosity was calculated as the proportion of loci for which segregation was observed.

Genetic map construction: Linkage maps were constructed with Mapmaker 3.0 (Lander *et al.* 1987; Lincoln *et al.* 1992) by completing several rounds of grouping and marker exclusion. For all calculations involving map distances we used the Kosambi mapping function. Marker grouping was initiated using the GROUP command with a LOD of 11.0 and a maximum mapping distance of 37 cM. The LOD threshold was then dropped in a stepwise fashion down to a LOD of 6.0, resulting in 22 putative linkage groups incorporating 331 markers in the *I. fulva* ($IbBC_1$) mapping population and 22 putative linkage groups incorporating 206 markers for the *I. brevicaulis* mapping population ($IfBC_1$'s). For each putative linkage group, all pairwise recombination fractions were scrutinized to ensure that no groups had been erroneously linked and to search for markers with conflicting linkage. In most cases a single anchor marker was designated for each linkage group using the ANCHOR command: in the case of the largest linkage groups, two anchor markers were used. Remaining loci were assigned to linkage groups using the ASSIGN command at a LOD of 5.0 and a maximum mapping

distance of 37.0 cM. Linkage of any loci remaining unassigned was examined at decreasing LOD thresholds, holding the maximum mapping distance constant at 37.0 cM. Markers that could be unambiguously assigned to groups at lower LOD thresholds were kept in the data set; those showing conflicting linkage were discarded.

The marker order for each linkage group was established using the ORDER command of MAPMAKER with error detection on. The ORDER command was set to search for an initial order of four markers in most cases (three for smaller groups, five for larger groups) with a support-for-order LOD threshold of 3.0. Remaining markers were added to this initial order first at a LOD of 3.0 and then at a LOD of 2.0. Resulting marker orders were then examined using the RIPPLE command. In a few cases, marker orders were established with lower thresholds. Candidate errors identified by the ERROR DETECTION capability of the ORDER function were rescored, and loci with a high number of errors that could not be resolved were excluded from the framework map or discarded. The ordering procedure described above was then repeated after correcting genotyping errors and excluding high-error markers from the framework map. After this second round of ordering, the framework marker order for each linkage group was set using the FRAMEWORK command, and remaining markers were placed in their most likely interval using the PLACE function.

Marker spacing, genome length, and coverage estimation: The average framework marker spacing, *s*, was calculated by dividing the summed length of all linkage groups by the number of intermarker intervals (equal to the number of framework markers minus the number of linkage groups) (Fishman *et al.* 2001). The total genome length, *L*, was estimated using two different methods to account for chromosome ends beyond the terminal markers of each linkage group. The first method added 2*s* to the length of all linkage groups. The second method, method 4 of Chakravarti *et al.* (1991), was employed by multiplying the length of each linkage group by the factor $(m+1)/(m-1)$, where m is the number of framework markers on each group. The map coverage, *c*, was estimated as the percentage of the genome within *d* cM of a framework marker, where $c = 1 - e^{(-2dn/L)}$, and n is the number of framework markers (Chakravarti *et al.* 1991).

Following the method employed by Cervera *et al.* (2001), a coefficient of dispersion was calculated (Sokal and Rohlf 1995) to address whether *IRRE* display markers appear clustered in the linkage map. If the occurrence of an *IRRE* insertion site and marker locus is assumed to be a rare, independent event, then the frequency distribution of the number of marker loci occurring in each 10-cM interval should follow a Poisson distribution. To test this, the linkage map was divided into 10-cM intervals, and the number of intervals with 0–15 markers was counted. The coefficient of dispersion was calculated as $CD = s^2/Y$, where Y is the mean number of markers per 10-cM interval, and s^2 is the sample variance. A value of CD >1.0 indicates that loci are more clumped than would be expected if the occurrence of *IRRE* display loci are random and independent; a value <1.0 indicates that loci are more dispersed than would be expected.

Comparison to expected segregation ratios: Agreement with expected Mendelian segregation ratios was evaluated using the χ^2 goodness-of-fit test with the degrees of freedom equal to the number of observed genotypic classes (*e.g.*, 2.0) minus 1.0 (Sokal and Rohlf 1995). Observed genotype frequencies of all marker loci incorporated into the linkage map were compared to expected genotype frequencies segregating in a 1:1 ratio. Each locus was tested individually, and the presence of distorted map regions was evaluated on the basis of the map distribution of loci showing significant TRD. Bonferroni corrections accounting for multiple tests were not used because genotype frequencies at individual marker loci may be related by linkage and therefore should not be expected to vary independently. Our choice to not correct for multiple tests may lead us to erroneously reject the null hypothesis at some loci, due to the fact that a small number of our numerous tests would be expected to surpass our significance threshold due to chance alone. However, our approach is more likely to allow us to detect transmission ratio distortion in map regions where distortion might actually exist but is not extreme or where there is low marker coverage.

Detection of map regions showing transmission ratio distortion: To examine the pattern of TRD across the linkage map, we identified regions of two or more contiguous framework markers showing distortion in the same direction at $\alpha < 0.05$ and $\alpha < 0.01$. An indicator of the direction and magnitude of transmission ratio distortion was calculated as the relative frequency of the introgressed alleles: frequencies greater than or less than the expected value of 0.5 indicate over- or underrepresentation of the introgressed parent allele, respectively.

Bayesian mapping of transmission ratio distorting loci: The Bayesian multipoint mapping method of Vogl and Xu (2000) was implemented as an additional means of detecting and localizing map regions of transmission ratio distortion. This method treats the number, location, and effects of transmission ratio distorting loci (TRDLs) as unknowns and makes use of reversible jump Markov chain Monte Carlo (MCMC) to estimate these variables. For the MCMC procedure, a Poisson prior of zero and a maximum of four (or, in the case of small linkage groups, two) TRDLs per linkage group was used. The chain length was set to 3000 iterations, which was determined to be sufficient by performing trial runs using various chain lengths (data not shown). The resulting number of TRDLs, their positions, and effects were treated as samples from the joint posterior distribution. Each linkage group was analyzed separately, and only linkage groups containing at least three markers were analyzed.

RESULTS

Generation of transposon display genetic markers: A total of 48 *IRRE* transposon display primer combinations were tried in the $IbBC_1$ mapping population, 38 of which were also run in the $IfBC_1$ population (Table 1). Reactions based on the *Eco*RI + AG preselective primers were dropped because these reactions yielded fewer bands that were significantly less sharp than reactions based on *Eco*RI + AC or + CT primers. Transposon display reactions yield from 3 to 15 segregating If174-specific alleles (*If* alleles) and from 0 to 15 segregating Ib72-specific alleles (*Ib* alleles) (Figure 1). *IRRE1-C*- and *IRRE1-A1*-based reactions yield an average of 8.45 ($N=20$) and 8.75 ($N=28$) *If* alleles and 6.2 ($N=18$) and 10.2 ($N=20$) *Ib* alleles, respectively. The greater range in the number of *Ib* allele markers per reaction may be due in part to the higher heterozygosity (H_e) of the Ib72 parent. Overall heterozygosity, assessed for each parent by scoring segregation in progeny for which that genotype served as a recurrent parent, was 0.59 for Ib72 and 0.31 for If174.

Construction of the *I. fulva* linkage map using $IbBC_1$ hybrids: Linkage mapping of *I. fulva* using $IbBC_1$

TABLE 1

Segregating *IRRE* transposon display polymorphisms by primer combination

*Eco*RI primer selective extension	*IRRE-A1* element primer		*IRRE-C* element primer	
	I. fulva markers	*I. brevicaulis* markers	*I. fulva* markers	*I. brevicaulis* markers
CTTT	6	14	5	0
CTTG	5	11	7	7
CTTC	7	15	8	8
CTTA	5	9	11	10
CTGT	15	14	7	10
CTGA	6	11	6	5
CTCT	7	10	8	7
CTCA	10	8	10	9
CTAT	13	10	8	11
CTAG	6	11	8	6
CTAC	12	12	13	14
CTAA	9	12	14	8
AGTT	6	—	—	—
AGTG	3	—	—	—
AGTC	8	—	—	—
AGTA	10	—	—	—
AGCA	8	—	—	—
AGAT	5	—	—	—
AGAC	8	—	—	—
AGAA	7	—	—	—
ACTT	13	4	7	1
ACTC	8	11	8	0
ACTA	14	9	8	2
ACGT	10	7	6	1
ACGA	14	9	8	—
ACAT	9	12	7	6
ACAC	9	6	12	1
ACAA	12	6	8	—
Total	245	201	169	106

hybrids was based on a data set initially consisting of 414 *IRRE* transposon display markers genotyped in a subset of 120 progeny. Preliminary linkage analysis identified a group of 148 framework markers, which were then genotyped in the remaining 110 progeny subsequent to the final round of linkage map construction. A total of 401 markers were incorporated into the final *I. fulva* linkage map (Figure 2). The majority (344) of the 414 markers in the initial data set were assigned to linkage groups at LOD = 5.0 at a maximum mapping distance of 37.0 cM. Linkage grouping of the remaining 70 loci was completed at lower LOD thresholds, holding the maximum mapping distance constant at 37.0 cM. Seventeen loci were assigned to linkage groups at a LOD of 4.0, 14 were assigned at a LOD of 3.0, 18 assigned at a LOD of 2.0, and 8 at a LOD of 1.5. Finally, 6 markers were discarded due to conflicting linkage, 6 markers demonstrated linkage to groups at LODs <1.5 but were discarded, and 1 marker was completely unlinked.

The *I. fulva* linkage map consists of 142 framework markers on 22 linkage groups (Figure 2). Framework markers were those that could be ordered at a multipoint linkage analysis LOD threshold of at least 2.0. A total of 259 additional markers have been assigned to linkage groups and placed on the map but could not be assigned to a single interval at this statistical threshold.

The number of linkage groups resolved (22) agrees well with the cytologically determined haploid chromosome number of 21 (Randolph *et al.* 1961). The number of framework markers per linkage group ranges from 2 to 14, and linkage groups vary in length from 12 to 148 cM (Figure 2). The total length of all linkage groups resolved in the *I. fulva* linkage map is 1439 cM. The average marker spacing is 12 cM. Estimates of the total map length range from 1966 cM, calculated by adding 2*s* to each of the 22 linkage groups, to 1980 cM, calculated using method 4 of Chakravarti *et al.* (1991). This results in a coverage estimate of 76% of the genome within 10 cM of a framework marker. The coefficient of dispersion of 3.22 indicates that markers are clustered.

Construction of the *I. brevicaulis* linkage map using IfBC$_1$ hybrids: A total of 286 markers were incorporated into the *I. brevicaulis* linkage map (Figure 3). The majority (215) of the 307 markers in the initial data set were assigned at a LOD of 5.0 and a maximum mapping distance of 37.0 cM. Linkage of the remaining 92 loci was examined at decreasing LOD thresholds, holding the maximum mapping distance constant at 37.0 cM. Eleven loci were assigned to linkage groups at a LOD of 4.0, 31 were assigned at a LOD of 3.0, 15 assigned at a LOD of 2.0, and 14 at a LOD of 1.5. Finally, 13 markers were discarded due to conflicting linkage, 3 markers demonstrated linkage to groups at LODs <1.5 but were discarded, and 5 markers were unlinked.

The final *I. brevicaulis* linkage map consists of 106 framework markers on 22 linkage groups (Figure 3). A total of 180 additional markers were placed on the map but could not be assigned to a single interval at our statistical thresholds (multipoint order support of an LOD at least 2.0).

The number of linkage groups resolved (22) is one greater than the 21 haploid chromosomes reported for *I. brevicaulis* (Randolph *et al.* 1961), but is equal to the number of linkage groups resolved in the *I. fulva* linkage map (discussed above). The number of framework markers per linkage group ranges from 2 to 10, and linkage groups vary in length from 0.0 to 166 cM (Figure 3). The total length of all linkage groups resolved in the *I. brevicaulis* linkage map is 1108.6 cM. The average marker spacing is 13.0 cM. Estimates of the total map length range from 1708.5 cM, calculated by adding 2s to each of the 22 linkage groups, to 1604.4 cM, calculated using method 4 of Chakravarti *et al.* (1991). This results in a coverage estimate of 72–74% of the genome within 10 cM of a framework marker. The coefficient of dispersion of 2.80 indicates that markers are clustered.

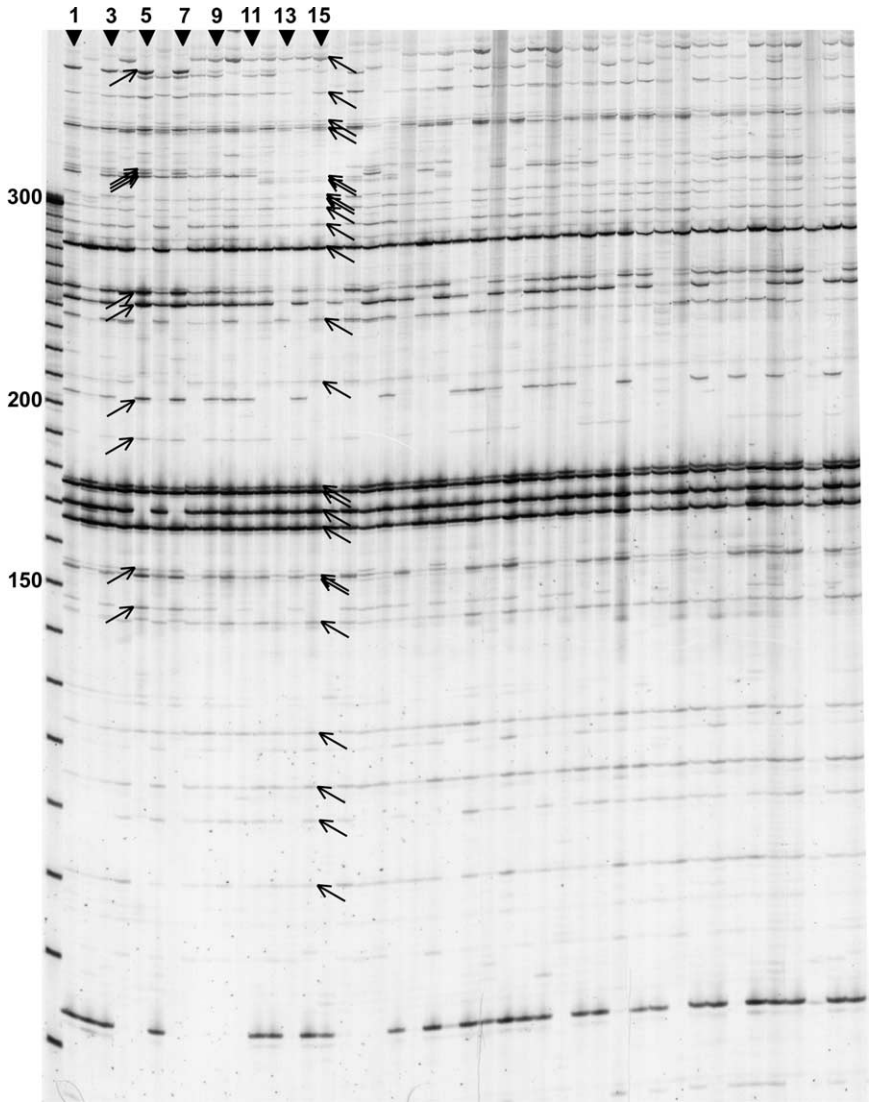
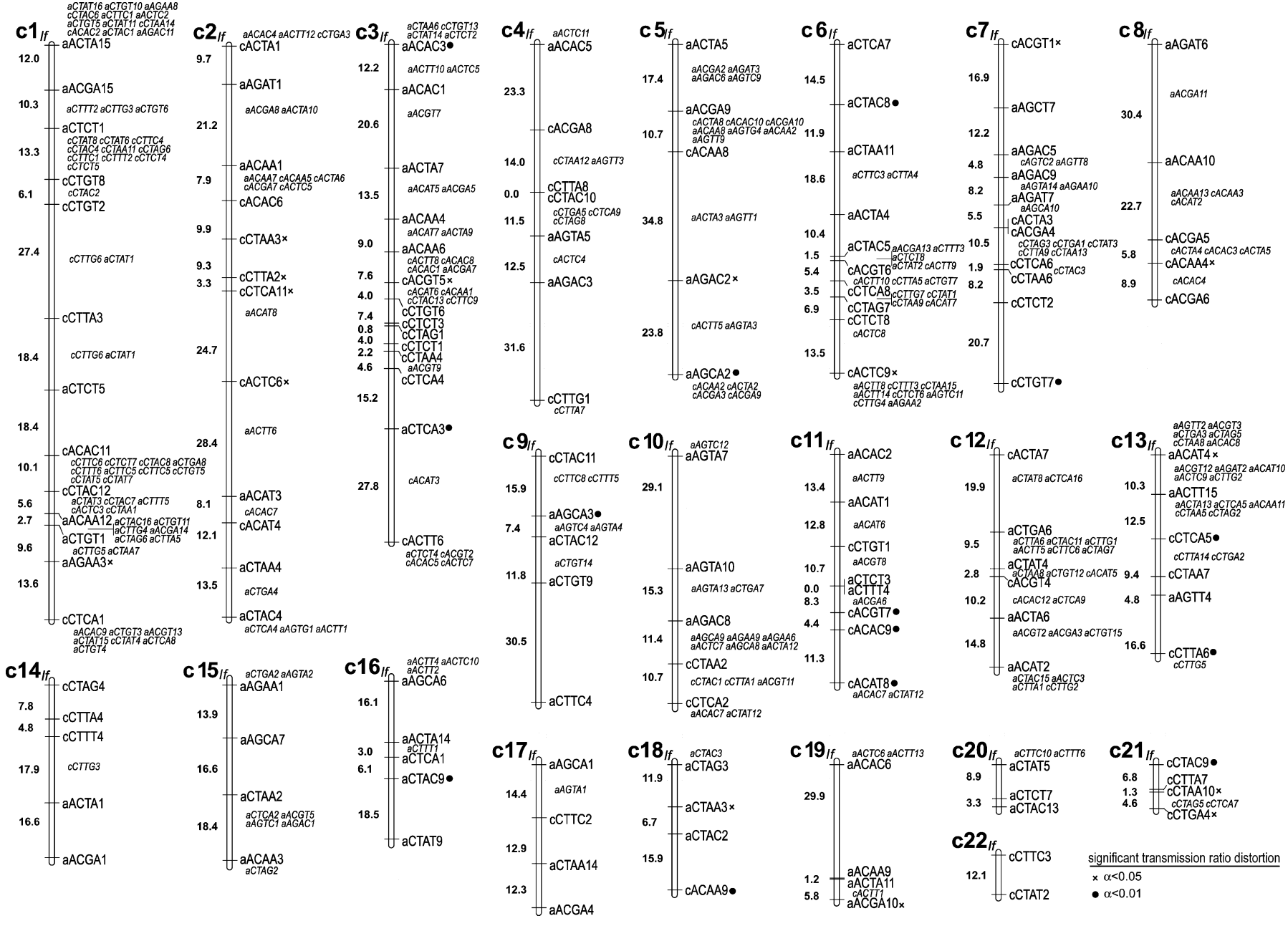


FIGURE 1.—Example of *IRRE* transposon display markers segregating in $IbBC_1$ hybrids. Replicate samples of the introgressed If174 parent are in lanes 5 and 7, replicate samples of the $F_1(3)$ parent are in lanes 9 and 11, and replicate samples of the recurrent Ib72 parent are in lanes 13 and 15. $F_1(2)$ is in lanes 1 and 3; remaining lanes are $IbBC_1$ hybrids. Examples of segregating If174 allele markers used in linkage mapping of *I. fulva* are indicated by arrows in lane 5. Examples of Ib72 alleles scored for estimating observed heterozygosity are indicated by arrows in lane 15. Size markers to the left are in base pairs.

Little transmission ratio distortion is evident in markers segregating in backcross hybrids between *I. fulva* and *I. brevicaulis*: A minority of *If* allele markers segregating in $IbBC_1$ hybrids shows deviation from Mendelian expectation. Of the 401 markers in the map, 15.7% show distortion at the $\alpha = 0.05$ significance level. The majority (71.9%) of these show an overrepresentation of the introgressed If174 allele. From examining the pattern of distorted loci on the linkage map, five map regions (linkage groups 2, 5, 6, 11, and 21) appear to contain putative TRDLs on the basis of our criteria of at least 2 contiguous distorted framework markers (Figure 4A). All but one of these map regions (linkage group 21) are areas of overrepresentation of the introgressed *If* allele. Only 1 of the 13 markers that were discarded during the mapping process showed statistically significant TRD.

A similar minority of the *Ib* allele markers segregating in the $IfBC_1$ hybrids shows deviation from Mendelian expectation. Of the 286 markers in the map, 15.3% show distortion at the $\alpha = 0.05$ significance level. As in the *I. fulva* mapping analysis, the majority (56.8%) of distorted markers incorporated into the *I. brevicaulis* linkage map show an overrepresentation of the introgressed

FIGURE 2.—Linkage map of *I. fulva IRRE* retrotransposon display markers segregating in $IbBC_1$ hybrids. The first letter of each marker name (a or c) indicates *IRRE-A1* and *IRRE-C* marker loci, respectively. The last four letters in each marker name indicate the *Eco*RI primer combination used to resolve the locus. Framework marker placements are indicated by a horizontal slash. Placed markers are shown in their most likely interval of location and are indicated by italics. Intermarker recombination distances are shown in Kosambi cemtimorgans. Markers showing statistically significant transmission ratio distortion are designated by x ($\alpha < 0.05$) or a solid circle ($\alpha < 0.01$).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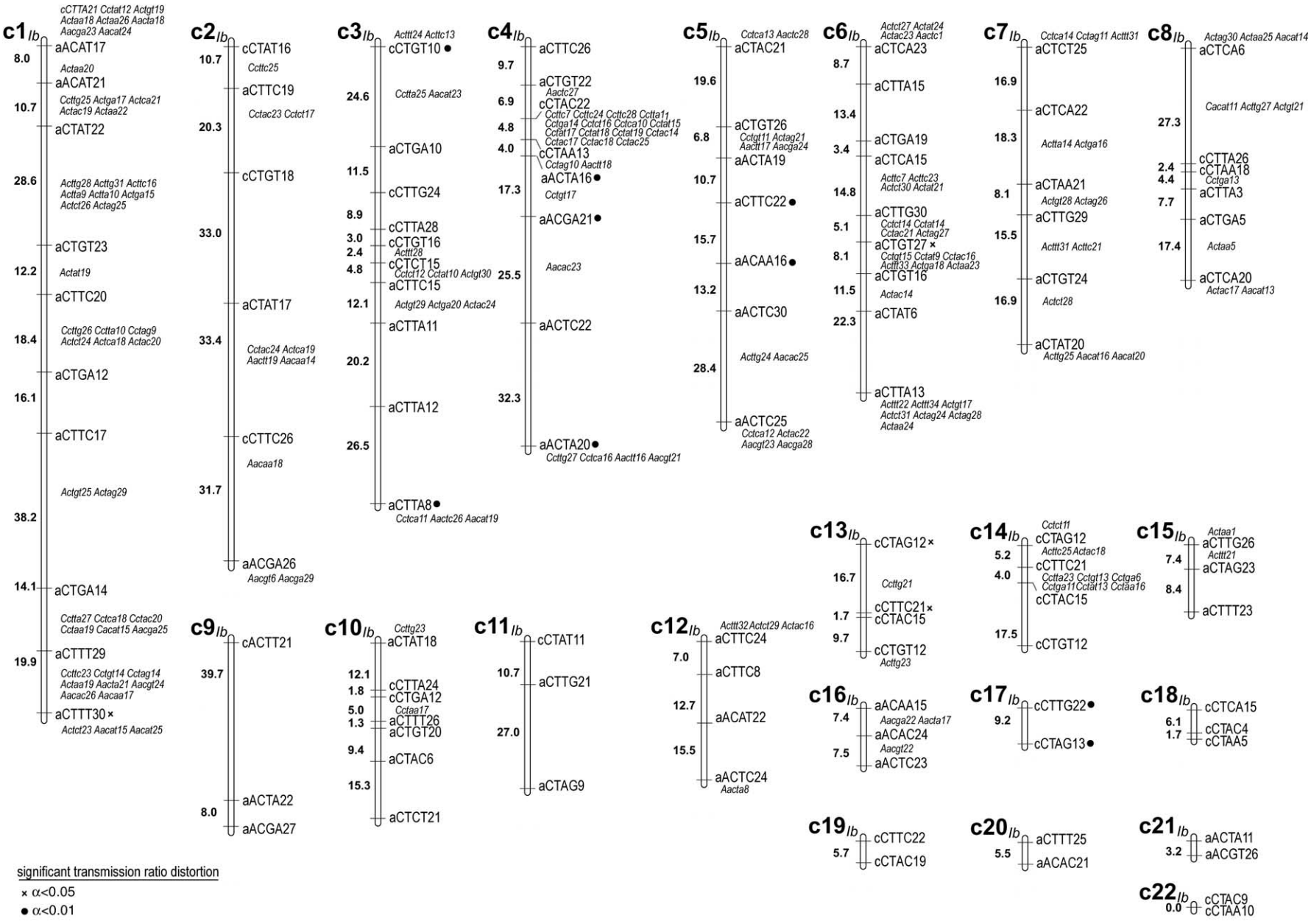
aACTA15
12.0
aACGA15
10.3
aCTTT2 aCTTG3 aCTGT6
aCTCT1
13.3
cCTAT8 cCTAT6 cCTTC4 cCTAC4 cCTAA11 cCTAG6 cCTTC1 cCTTT2 cCTCT4 cCTCT5
cCTGT8
6.1
cCTAC2
cCTGT2
27.4
cCTTG6 aCTAT1
cCTTA3
18.4
cCTTG6 aCTAT1
aCTCT5
18.4
cACAC11
10.1
cCTTC6 cCTCT7 cCTAC8 aCTGA8 cCTTT6 aCTTC5 cCTTC5 cCTGT5 cCTAT5 cCTAT7
cCTAC12
5.6
aCTAT3 cCTAC7 aCTTT5 cACTC3 cCTAA1
2.7
aCAA12
aCTAC16 aCTGT11 aCTTG4 aCGA14 aCTAG6 aCTTA5
aCTGT1
9.6
aCTTG5 aCTAA7
aAGAA3×
13.6
cCTCA1
aACAC9 aCTGT3 aACGT13 aCTAT15 cCTAT4 aCTCA8 aCTGT4
c2 lf
aACAC4 aACTT12 cCTGA3
9.7
cACTA1
aAGAT1
aACGA8 aACTA10
21.2
aACAA1
aACAA7 cACAA5 cACTA6 cACGA7 cACTC5
7.9
cACAC6
9.9
cCTAA3×
9.3
3.3
cCTTA2×
cCTCA11×
aACAT8
24.7
cACTC6×
aACTT6
28.4
8.1
aACAT3
cACAC7
cACAT4
12.1
aCTAA4
aCTGA4
13.5
aCTAC4
aCTCA4 aAGTG1 aACTT1
c3 lf
aCTAA6 cCTGT13 aCTAT14 aCTCT2
aACAC3●
12.2
aACTT10 aACTC5
aACAC1
aACGT7
20.6
aACTA7
aACAT5 aACGA5
13.5
aACAA4
aACAT7 aACTA9
9.0
aACAA6
cACTT8 cACAC8 cACAC1 aACGA7
7.6
cACGT5×
4.0
cACAT6 cACAA1 cCTAC13 cCTTC9
7.4
cCTGT6
0.8
cCTCT3
4.0
cCTAG1
2.2
cCTCT1
4.6
cCTAA4
aACGT9
15.2
cCTCA4
aCTCA3●
27.8
cACAT3
cACTT6
aCTCT4 cACGT2 cACAC5 cACTC7
c4 lf
aACTC11
aACAC5
23.3
cACGA8
14.0
cCTAA12 aAGTT3
cCTTA8
0.0
cCTAC10
11.5
cCTGA5 cCTCA9 cCTAG8
aAGTA5
12.5
cACTC4
aAGAC3
31.6
cCTTG1
cCTTA7
c5 lf
aACTA5
17.4
aACGA2 aAGAT3 aAGAC6 aAGTC9
aACGA9
10.7
cACTA8 cACAC10 cACGA10 aACAA8 aAGTG4 aACAA2 aAGTT9
cACAA8
34.8
aACTA3 aAGTT1
aAGAC2×
23.8
cACTT5 aAGTA3
aAGCA2●
cACAA2 cACTA2 cACGA3 cACGA9
c6 lf
aCTCA7
14.5
aCTAC8●
11.9
aCTAA11
18.6
cCTTC3 aCTTA4
aACTA4
10.4
aCTAC5
aACGA13 aCTTT3 aCTCT3 aCTAT2 aCTT9
1.5
5.4
cACGT6
cACTT10 cCTTA5 aCTGT7
3.5
cCTCA8
cCTTG7 cCTAT1 cCTAA9 cACAT7
6.9
cCTAG7
cCTCT8
cACTC8
13.5
aCTAC9×
aACTT8 cCTTT3 cCTAA15 aACTT14 cCTCT6 aAGTC11 cCTTG4 aAGAA2
c7 lf
cACGT1×
16.9
aAGCT7
12.2
aAGAC5
cAGTC2 aAGTT8
4.8
aAGAC9
aAGTA14 aAGAA10
8.2
aAGAT7
aAGCA10
5.5
cACTA3
10.5
cACGA4
cCTAG3 cCTGA1 cCTAT3 cCTTA9 cCTAA13
1.9
cCTCA6
cCTAC3
8.2
cCTAA6
cCTCT2
20.7
cCTGT7●
c8 lf
aAGAT6
aACGA11
30.4
aACAA10
aACAA13 cACAA3 cACAT2
22.7
5.8
cACGA5
cACTA4 cACAC3 cACTA5
cACAA4×
8.9
cACAC4
cACGA6
c9 lf
cCTAC11
cCTTC8 cCTTT5
15.9
aAGCA3●
7.4
aAGTC4 aAGTA4
aCTAC12
aCTGT14
11.8
aCTGT9
30.5
aCTTC4
c10 lf
aAGTC12
aAGTA7
29.1
aAGTA10
15.3
aAGTA13 aCTGA7
aAGAC8
11.4
aAGCA9 aAGAA9 aAGAA6 aACTC7 aAGCA8 aACTA12
cCTAA2
10.7
cCTAC1 cCTTA1 aACGT11
cCTCA2
aACAC7 aCTAT12
c11 lf
aACAC2
aACTT9
13.4
aACAT1
aACAT6
12.8
cCTGT1
aACGT8
10.7
aCTCT3
0.0
aCTTT4
8.3
aACGA6
aCACG7●
4.4
aCACA9●
11.3
aCACA8●
aACAC7 aCTAT12
c12 lf
aCACTA7
aCTAT8 aCTCA16
19.9
aCTGA6
aCTTA6 aCTAC11 aCTTG1 aACTT5 aCTTC6 aCTAG7
9.5
aCTAT4
aCTAA8 aCTGT12 cACAT5
2.8
cACGT4
10.2
cACAC12 aCTCA9
aACTA6
aACGT2 aACGA3 aCTGT15
14.8
aACAT2
aCTAC15 aACTC3 aCTTA1 cCTTG2
c13 lf
aAGTT2 aACGT3 aCTGA3 aCTAG5 cCTAA8 aACAC8
aACAT4×
aACGT12 aAGAT2 aACAT10 aACTC9 aCTTG2
10.3
aACTT15
aACTA13 aCTCA5 aACAA11 cCTAA5 cCTAG2
12.5
cCTCA5●
cCTTA14 cCTGA2
9.4
cCTAA7
4.8
aAGTT4
16.6
cCTTA6●
cCTTG5
c14 lf
cCTAG4
7.8
cCTTA4
4.8
cCTTT4
17.9
cCTTG3
aACTA1
16.6
aACGA1
c15 lf
aCTGA2 aAGTA2
aAGAA1
13.9
aAGCA7
16.6
aCTAA2
aCTCA2 aACGT5 aAGTC1 aAGAC1
18.4
aACAA3
aCTAG2
c16 lf
aACTT4 aACTC10 aACTT2
aAGCA6
16.1
aACTA14
aCTTT1
3.0
aCTCA1
6.1
aCTAC9●
18.5
aCTAT9
c17 lf
aAGCA1
14.4
aAGTA1
cCTTC2
12.9
aCTAA14
12.3
aACGA4
c18 lf
aCTAC3
aCTAG3
11.9
aCTAA3×
6.7
aCTAC2
15.9
cACAA9●
c19 lf
aACTC6 aACTT13
aACAC6
29.9
aACAA9
1.2
aACTA11
aCACT1
5.8
aACGA10×
c20 lf
aCTTC10 aCTTT6
8.9
aCTAT5
3.3
aCTCT7
aCTAC13
c21 lf
6.8
cCTAC9●
1.3
cCTTA7
4.6
cCTAA10×
cCTAG5 cCTCA7
cCTGA4×
c22 lf
cCTTC3
12.1
cCTAT2
significant transmission ratio distortion
× α<0.05
● α<0.01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8.0
aACAT17
Actaa20
aACAT21
10.7
Ccttg25 Actga17 Actca21 Actac19 Actaa22
aCTAT22
28.6
Acttg28 Acttg31 Acttc16 Actta9 Actta10 Actga15 Actct26 Actag25
aCTGT23
12.2
Actat19
aCTTC20
18.4
Ccttg26 Cctta10 Cctag9 Actct24 Actca18 Actac20
aCTGA12
16.1
aCTTC17
Actgt25 Actag29
38.2
aCTGA14
14.1
Ccta27 Cctca18 Cctac20 Cctaa19 Cacat15 Aacga25
aCTTT29
19.9
Ccttc23 Cctgt14 Cctag14 Actaa19 Aacta21 Aacgt24 Aacac26 Aacaa17
aCTTT30×
Actct23 Aacat15 Aacat25
c2 lb
10.7
cCTAT16
Ccttc25
aCTTC19
20.3
Cctac23 Cctct17
cCTGT18
33.0
aCTAT17
33.4
Cctac24 Actca19 Aactt19 Aacaa14
cCTTC26
Aacaa18
31.7
aACGA26
Aacgt6 Aacga29
c3 lb
Acttt24 Acttc13
cCTGT10●
24.6
Cctta25 Aacat23
aCTGA10
11.5
cCTTG24
8.9
cCTTA28
3.0
cCTGT16
2.4
Acttt28
cCTCT15
4.8
Cctct12 Cctat10 Actgt30
aCTTC15
12.1
Actgt29 Actga20 Actac24
aCTTA11
20.2
aCTTA12
26.5
aCTTA8●
Cctca11 Aactc26 Aacat19
c4 lb
aCTTC26
9.7
aCTGT22
Aactc27
6.9
cCTAC22
4.8
Ccttc7 Ccttc24 Ccttc28 Cctta11 Cctga14 Cctct16 Cctca10 Cctat15 Ccfat17 Cctat18 Cctat19 Cctac14 Cctac17 Cctac18 Cctac25
4.0
cCTAA13
Cctag10 Aactt18
17.3
aACTA16●
Cctgt17
aACGA21●
25.5
Aacac23
aACTC22
32.3
aACTA20●
Ccttg27 Cctca16 Aactt16 Aacgt21
c5 lb
Cctca13 Aactc28
19.6
aCTAC21
6.8
aCTGT26
Cctgt11 Actag21 Aactt17 Aacga24
aACTA19
10.7
aCTTC22●
15.7
aACAA16●
13.2
aACTC30
Acttg24 Aacac25
28.4
aACTC25
Cctca12 Actac22 Aacgt23 Aacga28
c6 lb
Actct27 Actat24 Actac23 Aactc1
8.7
aCTCA23
aCTTA15
13.4
aCTGA19
3.4
aCTCA15
Acttc7 Acttc23 Actct30 Actat21
14.8
aCTTG30
5.1
Cctct14 Cctat14 Cctac21 Actag27
aCTGT27×
8.1
Cctgt15 Cctat9 Cctac16 Actft33 Actga18 Actaa23
aCTGT16
11.5
Actac14
aCTAT6
22.3
aCTTA13
Acttt22 Acttt34 Actgt17 Actct31 Actag24 Actag28 Actaa24
c7 lb
Cctca14 Cctag11 Acttt31
16.9
aCTCT25
aCTCA22
18.3
Actta14 Actga16
aCTAA21
8.1
Actgt28 Actag26
aCTTG29
15.5
Acttt31 Acttc21
aCTGT24
16.9
Actct28
aCTAT20
Acttg25 Aacat16 Aacat20
c8 lb
Actag30 Actaa25 Aacat14
aCTCA6
Cacat11 Acttg27 Actgt21
27.3
2.4
cCTTA26
4.4
cCTAA18
Cctga13
7.7
aCTTA3
aCTGA5
17.4
Actaa5
aCTCA20
Actac17 Aacat13
c9 lb
cACTT21
39.7
aACTA22
8.0
aACGA27
c10 lb
Ccttg23
12.1
aCTAT18
1.8
cCTTA24
5.0
cCTGA12
Cctaa17
1.3
aCTTT26
aCTGT20
9.4
aCTAC6
15.3
aCTCT21
c11 lb
cCTAT11
10.7
aCTTG21
27.0
aCTAG9
c12 lb
Acttt32 Actct29 Actac16
aCTTC24
7.0
aCTTC8
12.7
aACAT22
15.5
aACTC24
Aacta8
c13 lb
cCTAG12×
16.7
Ccttg21
1.7
cCTTC21×
cCTAC15
9.7
cCTGT12
Acttg23
c14 lb
Cctct11
5.2
cCTAG12
Acttc25 Actac18
4.0
cCTTC21
Ccta23 Cctgt13 Cctga6 Cctga11 Cctat13 Cctaa16
cCTAC15
17.5
cCTGT12
c15 lb
Actaa1
7.4
aCTTG26
Acttt21
8.4
aCTAG23
aCTTT23
c16 lb
7.4
aACAA15
Aacga22 Aacta17
aACAC24
7.5
Aacgt22
aACTC23
c17 lb
9.2
cCTTG22●
cCTAG13●
c18 lb
6.1
cCTCA15
1.7
cCTAC4
cCTAA5
c19 lb
cCTTC22
5.7
cCTAC19
c20 lb
aCTTT25
5.5
aACAC21
c21 lb
aACTA11
3.2
aACGT26
c22 lb
0.0
cCTAC9
cCTAA10
significant transmission ratio distortion
× α<0.05
● α<0.01

(Ib72) allele. On the basis of our criteria of at least 2 contiguous distorted framework markers four areas (linkage groups 4, 5, 13, and 17) appear to contain TRDLs (Figure 4B). Three of these (groups 4, 13, and 18) are areas of overrepresentation of the Ib72 allele, while one (group 5) is an area of underrepresentation of the Ib72 allele. Four (19%) of the 21 markers that were not included in the final linkage map showed statistically significant TRD.

The Bayesian MCMC method of Vogl and Xu (2000) was used as an additional method of mapping putative TRDLs. The posterior probability of a TRDL occurring in a given linkage group and the posterior distribution of TRDL positions were estimated from the results of 3000 MCMC iterations. For linkage groups identified as containing putative TRDLs on the basis of χ^2 tests (see above), the posterior probabilities for the presence of a TRDL ranged from 0.28 to 0.34 (Figure 4), with an average of 0.31. However, posterior probabilities for the presence of at least one TRDL on linkage groups for which no putative TRDLs were identified were also, on average, 0.31 (data not shown). Examination of the posterior distributions of TRDL locations indicates that the Bayesian MCMC procedure failed to conclusively localize TRDL in correspondence with regions of two or more distorted makers (Figure 4).

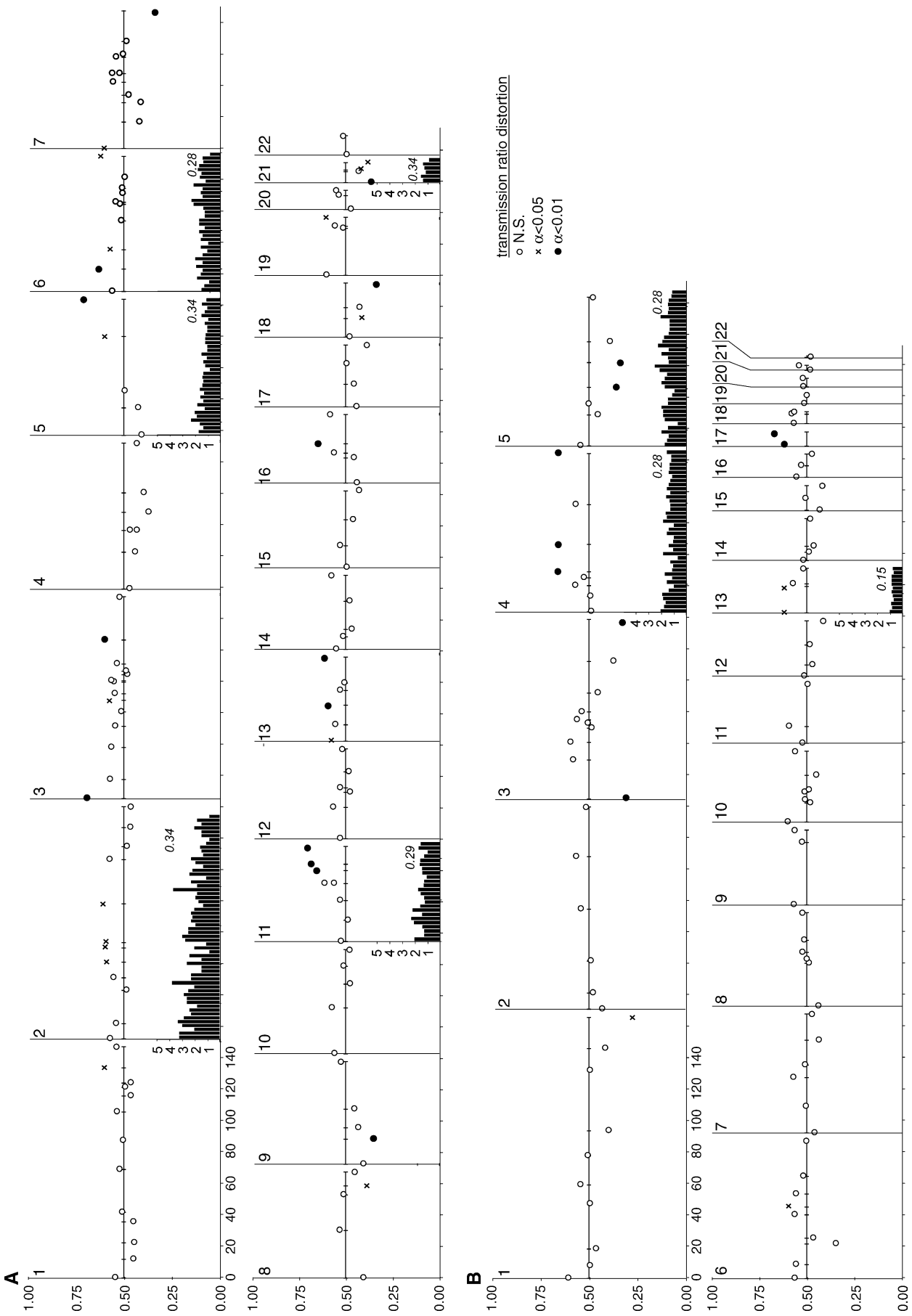
DISCUSSION

Genetic mapping of *I. fulva* and *I. brevicaulis* using *IRRE* retrotransposon display markers: Genetic maps of *I. fulva* and *I. brevicaulis* were constructed entirely with markers based on the *IRRE* LTR retroelement (Kentner *et al.* 2003). Mapping studies of other species, including barley, peas, and rice, have employed retroelement markers, but these studies incorporated retroelement-based markers into previously developed framework maps (Waugh *et al.* 1997; Ellis *et al.* 1998; Manninen *et al.* 2000; Jiang *et al.* 2002). This study appears to be the first case of constructing linkage maps *de novo* using retroelement-based transposon display markers in wild species. Total lengths of the *I. fulva* and *I. brevicaulis* maps presented here are estimated at 1966–1980 cM and 1604–1709 cM, respectively, resulting in coverage of 76 and 72% of the *I. fulva* and *I. brevicaulis* genomes within 10 cM of a marker. Unfortunately, the homology of linkage groups in the *I. fulva* and *I. brevicaulis* maps cannot be established with dominant *IRRE* retrotransposon markers alone as analyzed here. Both maps had a coefficient of dispersion >1.0, indicating that *IRRE* transposon display markers are clustered. Clustering and nesting of retrotransposons has been reported in other genetic and physical mapping studies (Waugh *et al.* 1997; SanMiguel *et al.* 1998; Kumekawa *et al.* 1999; Shirasu *et al.* 2000). However, our results indicate that *IRRE* transposon display markers provide map coverage adequate for initial genetic and QTL mapping studies. These results demonstrate the utility of transposon display markers for studies of species with few genetic marker resources or with large genomes that can prove intractable for standard marker methods like microsatellites and AFLPs.

The mapping populations were generated from two wild-collected, heterozygous individuals, and this may have had consequences for the construction of the maps. The higher H_e of 0.59 for Ib72 caused us to have a lower number of Ib72 bands inherited by and segregating from the $F_1(2)$ $IfBC_1$ parent, as compared with the number of If174 bands segregating in the $IbBC_1$ hybrids (If174 $H_e = 0.31$). This resulted in a smaller data set for construction of the *I. brevicaulis* linkage map as compared to the *I. fulva* map (Table 1). The *I. brevicaulis* map both is shorter and has less coverage than the *I. fulva* linkage map (1709 cM *vs.* 1980 cM and 72% *vs.* 76% coverage), and it includes five linkage groups composed of just two marker loci each. Other genetic mapping studies of wild species used cultivated inbred lines as recurrent parents (Rieseberg *et al.* 1995; Kim and Rieseberg 1999; Burke *et al.* 2002) or made inbred lines before initiating the mapping crosses (Fishman *et al.* 2001). Such approaches are likely to increase the success of mapping projects, but they are not always possible in studies of diverse wild plants such as iris, which takes over a year to begin flowering.

Genomes of both *I. fulva* and *I. brevicaulis* appear highly permeable to reciprocal introgression via backcrossing: Genetic mapping studies provide a means of assessing the number and distribution of genetic factors underlying species boundaries, including genomic regions contributing to hybrid fitness (Rieseberg *et al.* 1999). In linkage mapping experiments based on interspecific crosses, TRD at marker loci may indicate linkage to alleles conferring reduced or enhanced fertility, viability, or early survival in the hybrid mapping population (Paterson *et al.* 1991; Bernacchi and Tanksley 1997; Whitkus 1998; Rieseberg *et al.* 1999; Turelli and Orr 2000; Fishman *et al.* 2001), although the effects of meiotic drive systems cannot be ruled out. In this study, plants were genotyped after the mapping

Figure 3.—Linkage map of *I. brevicaulis IRRE* retrotransposon display markers segregating in $IfBC_1$ hybrids. The first letter of each marker name (a or c) indicates *IRRE-A1* and *IRRE-C* marker loci, respectively. The last four letters in each marker name indicate the *Eco*RI primer combination used to resolve the locus. Framework marker placements are indicated by a horizontal slash. Placed markers are shown in their most likely interval of location and are indicated by italics. Intermarker recombination distances are shown in Kosambi centimorgans. Markers showing statistically significant transmission ratio distortion are designated by x ($\alpha < 0.05$) or a solid circle ($\alpha < 0.01$).


A
B
transmission ratio distortion
○ N.S.
× α<0.05
● α<0.01
0.00
0.25
0.50
0.75
1.00
0 20 40 60 80 100 120 140
0.34
0.28
0.29
0.15

populations had been cultured in a greenhouse for ~18 months, meaning that map regions of TRD may reflect genotypic-specific differences in pollen viability or competition, embryo viability, germination rate, or seedling survival and establishment.

A minority of markers in both the *I. fulva* and the *I. brevicaulis* map show TRD (~15% in both maps; Figure 4), implying that little of either species' genome appears resistant to introgression from the other. Each of the maps includes only one map region (linkage group 21 on Figure 4A and linkage group 5 on Figure 4B) in which there appears to be a significantly reduced rate of introgression of heterospecific alleles. The linkage map of *I. fulva* includes an additional four regions in which the heterospecific alleles are overrepresented, and the linkage map of *I. brevicaulis* shows three such regions (Figure 4). The proportion of markers showing TRD is lower than is typically seen in interspecific backcross mapping experiments in which, on average, 30% of markers show TRD (Jenczewski *et al.* 1997), although a considerable range is observed. For example, 30 and 60% of marker loci mapped in interspecific backcrosses between species of Helianthus show TRD (Rieseberg *et al.* 1995; Kim and Rieseberg 1999). Paterson *et al.* (1988) reported that 69% of marker loci mapped in an interspecific backcross of *Lycopersicon esculentum* × (*L. esculentum* × *L. chmielewskii*) show TRD, while Bernacchi and Tanksley (1997) reported that 20% of marker loci exhibited TRD in a backcross of *L. esculentum* × (*L. esculentum* × *L. hirsutum*). An interspecific backcross of *Oryza sativa* × (*O. sativa* × *O. longistamina*) reported only 16% distorted marker loci (Causse *et al.* 1994).

Our χ^2 method of identifying map regions of TRD involved multiple tests. However, for both maps, the number of markers showing significant TRD is greater than the number that would be expected by chance alone. Of the *If* allele markers segregating in $IbBC_1$ hybrids, 30 and 14 framework loci showed TRD at the 0.05 and 0.01 significance levels, respectively, whereas only 7.1 and 1.42 would be expected by chance. Similarly, 13 and 9 of the framework loci in the *I. brevicaulis* linkage map showed significant TRD at the 0.05 and 0.01 significance levels, respectively, whereas 5.3 and 1.6 would be expected to do so by chance alone.

Results of the Bayesian MCMC procedure of Vogl and Xu (2000) also support the conclusion that few genetic incompatibilities affect introgression between *I. fulva* and *I. brevicaulis* and in fact imply fewer species barriers than do the results of the χ^2 method. Using a very conservative threshold for the detection of a TRDL (<50% of MCMC iterations resulting in 0 TRDL in a linkage group), only linkage group 18 in the *I. fulva* genetic map (Figure 4A) was found by the MCMC procedure to contain a TRDL (posterior probability of 0 TRDL = 0.4740). This linkage group did contain two loci that show statistically significant TRD using χ^2 tests (Figure 4A), but these markers were not contiguous. However, three of four markers on this linkage group have lower-than-expected frequencies of the introgressed allele, a pattern suggestive of the presence of a TRDL (Figure 4A). Linkage groups that did contain two or more contiguous distorted markers did not have correspondingly higher posterior probabilities for containing TRDLs than linkage groups that did not have contiguous distorted markers (average posterior probabilities for the presence of a TRDL = 0.31 for both). Previous studies employing the Bayesian MCMC procedure of Vogl and Xu (2000) reported generally good congruence between TRDLs mapped using this method and those identified by marker χ^2 tests (Fishman *et al.* 2001; Kuittinen *et al.* 2004; Myburg *et al.* 2004). However, these studies examined crosses in which both a greater proportion of markers were distorted and TRD was more severe than reported here in Iris (Fishman *et al.* 2001; Kuittinen *et al.* 2004; Myburg *et al.* 2004). In addition, the MCMC procedure is sensitive to mild deviations in expected allele frequencies, which occurs in many areas of the linkage maps presented here (C. Vogl, personal communication). Determining whether these deviations reflect linkage to genetic factors causing mild TRD would require mapping additional markers to better delimit these areas.

The low occurrence of TRD in the maps presented here is somewhat surprising, given the previous findings of skewed multilocus genotype frequencies indicating high seed abortion rates for certain hybrid classes (Cruzan and Arnold 1994) and the presence of significantly skewed multilocus genotype frequencies in experimental F_2 crosses (Burke *et al.* 1998). The differences in the results of this study and previous work may hinge on the differences in the genotypic classes surveyed and the degree of inbreeding used in the

Figure 4.—Observed frequencies of introgressed heterospecific alleles segregating in $IbBC_1$ (A) and $IfBC_1$ (B) hybrids. Each linkage group is represented by a graph and genetic distances are given on the *x*-axis in Kosambi centimorgans. Only framework marker loci are shown. Frequencies of the introgressed marker alleles are indicated on the *y*-axis (expected value is 0.50): Frequencies >0.50 indicate an overrepresentation of the introgressed alleles, while frequencies <0.50 indicate an underrepresentation of the introgressed alleles. Marker transmission ratio distortion as evaluated by χ^2 tests is indicated by an open circle (nonsignificant), x ($\alpha < 0.05$), and a solid circle ($\alpha < 0.01$). For linkage groups encompassing two or more contiguous markers showing significant transmission ratio distortion, the Bayesian posterior probability of a TRDL is given in italics and the posterior distribution of TRDL location is shown as an accompanying histogram (*y*-axis is the percentage of MCMC runs localizing the TRDL in 5-cM intervals).

experimental crossing designs. Burke *et al.* (1998) documented a significant underrepresentation of some multilocus genotypes in outcrossed F_2 hybrid seedlings of *I. fulva* and *I. brevicaulis*. Genotypes present in F_2 hybrids would include those subject to breakdown due to both recessive and dominant-negative epistatic interactions between *I. fulva* and *I. brevicaulis* alleles. Theory predicts that F_2 hybrids should be subject to more hybrid breakdown than BC_1 hybrids (Turelli and Orr 2000). Cruzan and Arnold's (1994) work involved surveying multilocus genotype frequencies at the embryonic stage in a natural hybrid zone population using dominant RAPD markers. It is unknown what the genetic pedigree of Cruzan and Arnold's (1994) plants were: aborted genotypes may have indeed been homozygous for alleles from different species at different loci, leading to hybrid breakdown in viability and the observed skew in multilocus genotype frequencies.

In this study, most of the markers showing TRD do so because of an overrepresentation of introgressed alleles (Figure 4). In contrast to this result, it is generally expected that the introgression of heterospecific alleles should have negative fitness consequences (Rieseberg and Carney 1998), resulting in their occurrence at lower-than-expected frequencies. However, other mapping studies of interspecific crosses have also found areas in which rates of introgression appear to be accelerated by an overrepresentation of heterospecific alleles. In a study of a BC_1 between two species of tomato, Bernacchi and Tanksley (1997) reported a total of nine genetic map regions exhibiting TRD, six of which were due to an overrepresentation of the introgressed parent alleles. A study of reciprocal BC_1 crosses between two species of Eucalyptus reported that half of the regions of TRD in both maps were due to overrepresentation of introgressed alleles (Myburg *et al.* 2004), leading these authors to conclude that heterospecific alleles had positive effects on hybrid fitness, which caused rates of introgression to be accelerated in some map regions. Studies of backcross hybrids in Helianthus have also detected genetic map regions in which introgressed alleles are overrepresented, although the majority of TRD is due to underrepresentation of the introgressing alleles (Rieseberg *et al.* 1995, 1996).

It is important to recognize that the overrepresentation of heterospecific alleles in both hybrid populations presented here could in fact reflect selection against homozygosity in some map regions due to inbreeding depression caused by our experimental design. Our crossing design, in which the same genotypes were used as both grandparents and parents of the BC_1 hybrids, was designed to minimize the amount of genetic variation segregating in the mapping populations and thus to maximize the future potential use of these populations for QTL mapping studies. However, this also means that our results might be confounded by inbreeding depression in the BC_1 hybrids: map regions in which introgressed parent alleles are overrepresented may be simply due to selection against genotypes that are homozygous for the recurrent parent alleles. In other words, because the fitness effects of introgressed alleles might be modulated by the fitness effects of inbreeding in our study, we may have underestimated the number of genomic regions contributing to species barriers in natural, outcrossing hybrid populations. More crossing experiments would need to be done to evaluate this possibility.

Implications for the evolutionary dynamics of hybridization between *I. fulva* and *I. brevicaulis*: Generalizations based on the results presented here must be made with caution until the completion of additional crossing experiments and map-based investigations of wild hybrid populations (Rieseberg *et al.* 1999). As in most genetic mapping studies, the results reported here may be specific to the individual genotypes crossed. Further, the fact that *IRRE* transposon display markers are highly polymorphic in both *I. fulva* and *I. brevicaulis* populations (reported in Kentner *et al.* 2003 at 45 and 63% for *IRRE-A1* and *IRRE-C* in *I. fulva* and at 84 and 82% for *IRRE1-A1* and *IRRE1-C* in *I. brevicaulis*) will present a challenge for the extension of these results to studies of wild hybrid populations until the conversion of mapped loci to species-specific codominant markers can be completed. However, given the potential for natural hybridization and introgression to contribute to adaptation and speciation, determining which portions of hybridizing plant genomes are permeable to introgression and which are resistant is important for understanding the possible outcomes of hybridization (Rieseberg *et al.* 1995, 2000; Arnold 2004a,b).

Overall, the results of both methods employed to analyze TRD suggest that little of either species' genome should show nonneutral introgression in natural hybrid zones due to intrinsic genetic incompatibilities alone. Results of the χ^2 method were perhaps somewhat more conclusive in that this analysis identified a small number of genomic regions of TRD, providing a baseline for future investigations of introgression in natural populations. Actual patterns of introgression in hybrid zone populations of Louisiana Iris species are affected by a complex cascade of factors. These include ecological setting, timing of flowering, floral traits, pollinator behavior, gamete competition, and hybrid viability and fertility (Arnold *et al.* 1993; Carney *et al.* 1994; Emms *et al.* 1996; Burke *et al.* 1998; Emms and Arnold 2000; Wesselingh and Arnold 2000; Johnston *et al.* 2001a, 2003). Since all these factors interact, genetic covariance between numerous traits might be important in preventing or facilitating gene flow between *I. fulva* and *I. brevicaulis* in natural settings. Future investigations will examine the presence of genetic covariance among hybrid fitness, ecological traits, and floral morphology, as well as their effects on fitness in experimental hybrid populations.

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