Copyright © 1995 by the Genetics Society of America

Chromosomal and Genic Barriers to Introgression in Helianthus

Loren H. Rieseberg,* C. Randal Linder* and Gerald J. Seiler†

Department of Biology, Indiana University, Bloomington, Indiana 47405 and †USDA-ARS, Northern Crop Science Laboratory, Fargo, North Dakota 58105

Manuscript received June 2, 1995
Accepted for publication August 17, 1995

ABSTRACT

The sexual transfer of genes between taxa possessing different structural karyotypes must involve the passage of genes through a chromosomal sterility barrier. Yet little is known about the effects of structural differences on gene introgression within or adjacent to the rearranged chromosomal fragments or about the patterns of introgression in collinear regions. Here, we employ 197 mapped molecular markers to study the effects of chromosomal structural differences on introgression in backcrossed progeny of the domesticated sunflower, *Helianthus annuus*, and its karyotypically divergent wild relative, *H. petiolaris*. Forty percent of the genome from the seven collinear linkages introgressed, whereas only 2.4% of the genome from the 10 rearranged linkages was transferred. Thus, chromosomal rearrangements appear to provide an effective mechanism for reducing or eliminating introgression in rearranged chromosomal segments. On the other hand, observations that 60% of the markers from within the collinear portion of the genome did not introgress suggests that genic factors also resist introgression in Helianthus. That is, selection against *H. petiolaris* genes in concert with linkage may have reduced or eliminated parts of the genome not protected by structural changes. Thus, barriers to introgression in Helianthus appear to include both chromosomal structural and genic factors.

FEW areas of plant evolutionary biology have attracted more interest and discussion than introgressive hybridization and its evolutionary significance. Some botanists have held the view that introgression is a potent evolutionary force, fostering the acquisition or development of novel adaptations (*e.g.*, ANDERSON 1949; STEBBINS 1950; RAVEN 1976; GRANT 1981). In contrast, others have accorded little evolutionary significance to introgression suggesting instead that it should be considered a primarily local phenomenon with only transient effects, a kind of "evolutionary noise" (BARBER and JACKSON 1957; RANDOLPH *et al.* 1967; WAGNER 1969, 1970; HARDIN 1975). Until recently, however, it has been difficult to evaluate these divergent views, because supportive evidence for introgression often has had alternative explanations (GOTTLIEB 1972; HEISER 1973).

This situation has changed dramatically during the past decade. A major contributing factor has been technological advances in molecular biology that have provided students of hybridization access to an almost unlimited number of markers (HARRISON 1990). These molecular markers have greatly enhanced our ability to detect and quantify introgression (*e.g.*, KEIM *et al.* 1989; ARNOLD *et al.* 1990, 1991; WHITTEMORE and SCHAAL 1991; BRUBAKER *et al.* 1993), with perhaps a less dramatic influence on our ability to perceive its evolutionary consequences. Equally important have been molecular phylogenetic studies that have detected many unexpected instances of hybridization and introgression (*e.g.*, DOEBLEY 1989; SMITH and SYTSMA 1990; RIESEBERG *et al.* 1991; BRUNSFELD *et al.* 1992). These studies indicate that introgression is widespread both geographically and phylogenetically in plants, generating renewed interest in the subject and stimulating several recent reviews (*e.g.*, RIESEBERG and SOLTIS 1991; RIESEBERG and BRUNSFELD 1992; ABBOTT 1992; ARNOLD 1992, 1994; RIESEBERG and WENDEL 1993). Most of these studies and reviews have focused on the extent, distribution, or consequences of hybridization and introgression, with less emphasis on the factors that promote hybridization or affect the passage of genes across a species barrier (although see ARNOLD 1994; RIESEBERG and ELLSTRAND 1993). Yet these factors must be understood before any general rules or predictions can be made concerning hybridization and its consequences. This article focuses on one of the critical factors that influences the movement of genes or linkage groups across species barriers—chromosomal structural differences.

The sexual transfer of genes between populations possessing different structural karyotypes must involve the passage of genes through a chromosomal sterility barrier. This process has been modeled genetically (*e.g.*, STEBBINS 1971; GRANT 1981; JACKSON 1985; SYBENGA 1992) and the following basic conclusions can be made. First, structural differences between homologous chromosomes often lead to reduced pairing or unbalanced gametes in hybrids, and the hybrids sometimes exhibit

Corresponding author: Loren H. Rieseberg, Biology Department, Indiana University, Bloomington, IN 47405.
E-mail: lriesebe@sunflower.bio.indiana.edu

chromosomal sterility or semisterility (although see COYNE *et al.* 1993). This will have the effect of reducing map lengths and will disrupt or inhibit introgression within or adjacent to the rearranged chromosomal segments (HANSON 1959b). Second, although introgression should be reduced in these regions, given appropriate conditions of crossing over, most chromosomal sterility barriers (even those involving several chromosomes) should not be impermeable to gene flow (GRANT 1981; SYBENGA 1992).

Nonetheless, the importance of chromosomal factors in resisting introgression remains unclear. Part of the confusion relates to uncertainty regarding the effects of chromosomal rearrangements on hybrid fertility. The impact of chromosomal differences on introgression will be reduced if they do not lower hybrid fertility, and there are numerous reports in the literature of individuals heterozygous for chromosomal rearrangements that do not show the expected decrease in fertility (SITES and MORITZ 1987; COYNE *et al.* 1993). Furthermore, meiotic abnormalities are frequently observed in hybrids between species with nearly identical karyotypes, indicating that differences in genes can produce meiotic irregularities similar to those thought to result from chromosomal rearrangements. Additional confusion stems from patterns of introgression observed in nature. Narrow tension zones consisting entirely of first generation hybrids are sometimes observed for chromosomally divergent species (SEARLE 1993), whereas in other instances extensive introgression is reported (*e.g.*, ARNOLD *et al.* 1987; RIESEBERG *et al.* 1990, 1991; DORADO *et al.* 1992; PATTON 1993; SHAW *et al.* 1993), although its genomic distribution is unknown. It is noteworthy that comparable patterns are observed among taxa differentiated by genic factors alone (BARTON and HEWITT 1985). Thus, it has been difficult to convincingly demonstrate an important role for chromosomal rearrangements as reproductive barriers.

Fortunately, recent advances in the area of genetic linkage mapping allow detailed characterization of the effects of both chromosomal and genic factors on interspecific gene flow (RIESEBERG 1995b). Genetic linkage maps can be generated for hybridizing parental species so that the genomic location and linear order of molecular markers can be determined. The maps can then be compared to identify changes in gene order and by inference, the structural changes differentiating the parental species genomes. After completion of these maps, hybrid and introgressive individuals can be surveyed for the presence of mapped parental molecular markers. The resulting "graphical genotypes" (YOUNG and TANKSLEY 1989a) can be used to determine rates of introgression as well as the genomic distribution of introgressed genes. In particular, this approach allows rates and patterns of introgression in rearranged chromosomal segments to be compared with those from collinear genomic regions.

Here we employ genetic linkage mapping to describe the impact of chromosomal structural and genic differences on the genomic location and frequency of introgression between two chromosomally divergent species of sunflower, *Helianthus annuus* and *H. petiolaris.* Given that the majority of plant and animal species that have been analyzed karyotypically appear to differ in terms of chromosome structure (WHITE 1978; JACKSON 1985), the results should be relevant to most hybridizing species pairs.

MATERIALS AND METHODS

Plant materials: *H. annuus* and *H. petiolaris* are self-incompatible annuals, with haploid chromosome numbers of 17 (HEISER 1947). Comparative genetic linkage mapping (RIESEBERG *et al.* 1995) indicates that seven linkage groups are collinear between the two species, whereas the remaining 10 linkages differ structurally due to a minimum of seven interchromosomal translocations and three inversions (Figure 1). These structural changes generate multivalent formations and bridges and fragments in hybrids (HEISER 1947; CHANDLER *et al.* 1986), apparently leading to semisterility; F_1 pollen viabilities are typically less than 10% and seed set is less than 1% (HEISER 1947; CHANDLER *et al.* 1986). Nonetheless, the two species do hybridize in nature, and introgression has been reported (HEISER 1947; DORADO *et al.* 1992).

To analyze the effects of chromosomal rearrangements on the genomic location and rate of introgression, we generated a BC_2F_3 progeny between *H. annuus* and *H. petiolaris* (SEILER 1991) The initial interspecific cross was *H. annuus* (cmsHA89; female) × *H. petiolaris* subsp. *petiolaris* (PET-PET-1741-1; male). The F_1 hybrids were backcrossed twice to cmsHA89 and then selfed for two generations. Selfing was possible because the male parent provided nuclear restorer alleles and cultivated *H. annuus* is self-compatible. A minimum of 20 plants was used for each generation of backcrossing and selfing. Fifty-eight individuals from the BC_2F_3 progeny array were grown to a size sufficient for total DNA isolations.

DNA isolations: One gram of fresh leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle and then mixed with a CTAB extraction buffer (WHITKUS *et al.* 1992). The resulting slurry was filtered through a layer of miracloth (Calbiochem), and DNA was extracted following the method of DOYLE and DOYLE (1987), except that a second chloroform extraction was performed. Pelleted DNAs were dissolved in TE, further purified using the ELU-QUICK DNA Purification Kit (Schleicher and Schuell) and then quantified on a fluorometer.

RAPD marker surveys: Purified DNAs from the 58 BC_2F_3 progeny were surveyed for 197 random amplified polymorphic DNA (RAPD) markers (WILLIAMS *et al.* 1990) of known genomic location (RIESEBERG *et al.* 1995). The markers were amplified by 108 primers obtained from the University of British Columbia Biotechnology Laboratory (primers 101–500) and Operon Technologies (primer kits A-F) and cover over 84% (1157 cM) of the sunflower genome currently mapped (RIESEBERG *et al.* 1995b), with an average distance of 6.5 cM between markers based on *H. annuus* map distances.

RAPD amplifications followed the general procedure of WILLIAMS *et al.* (1990). The amplifications were carried out in a total volume of 25 μl starting with 1 μl (10 ng) of purified template DNA, 1 μl primer (15 ng), and a final concentration of 2 mM $MgCl_2$, 20 mM Tris-HCl, 100 μM each dNTP, and 1 U of *Taq* DNA polymerase. The reactions were overlaid with mineral oil and placed in an MJ Research Thermal Cycler

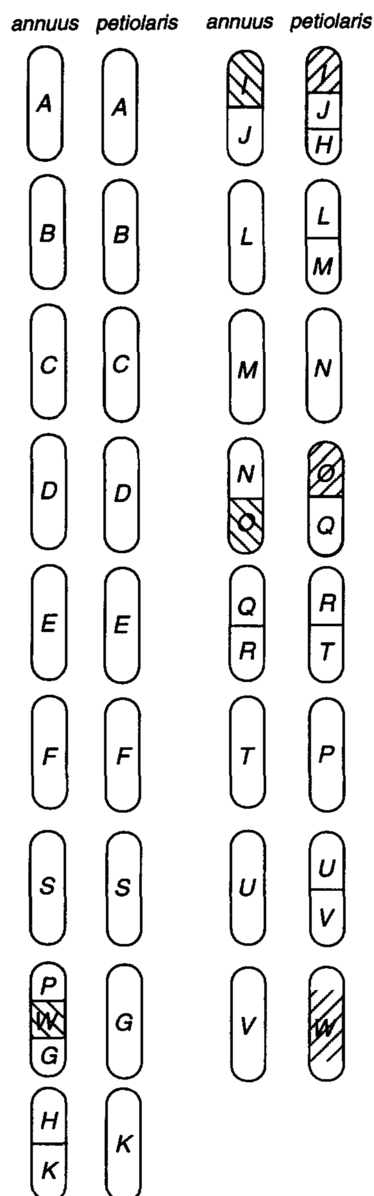

FIGURE 1.—Inferred chromosomal structural relationships for *Helianthus annuus* and *H. petiolaris* based on comparative linkage mapping. Hashed lines within linkages indicate inversions.

programmed for 45 cycles of 1 min at 94°, 1 min at 36°, and 2 min at 72° followed by a final extension at 72° for 7 min. Amplification products were separated by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide.

Graphical genotype construction: Upon completion of the marker survey, introgressed markers of the donor parent (*H. petiolaris*), were plotted onto the genomic map of the recipient species (*H. annuus*), generating a graphical genotype (YOUNG and TANKSLEY 1989a) for each of the 58 backcrossed progeny (Figure 2). The graphical genotype is based on the 1084-cM genomic map of the recipient species, *H. annuus* (RIESEBERG *et al.* 1995), which has been extended by approximately 290 cM due to the occurrence of several *H. petiolaris* markers outside currently mapped regions in *H. annuus* (homology tests and alignment of linkage maps from both species, as well as their hybrid derivative, *H. anomalus,* are described by RIESEBERG [1995a] and RIESEBERG *et al.* [1995], respectively). The presence of two adjacent introgressed markers on the graphical genotype of a single individual was taken as evidence that the entire fragment between the markers was derived from *H. petiolaris* through introgression. However, because the RAPD markers employed here are largely dominant, we often were unable to determine the linkage phase of adjacent markers. As a result, the possibility that a small proportion of adjacent introgressed markers are in repulsion phase (*i.e.*, on different homologues) cannot be ruled out, and the size of the introgressed segment(s) in these situations might be considerably smaller. Likewise, because of dominance, we were unable to determine whether the introgressed markers or fragments were homozygous or heterozygous.

Data analysis: Contingency table analysis was used to test whether the number of introgressed markers in the collinear and rearranged portions of the genome differed from expectations for dominant loci in a BC_2F_3 progeny with no barriers to introgression (15.6% on average). However, to determine whether there was significant heterogeneity in rates of marker introgression within each of these genomic regions, 100 simulations of unrestricted introgression for the collinear and rearranged portions of the genome were performed. For each simulation of a portion of the genome we randomly sampled the proportion of markers expected to introgress from the total markers assayed for that portion for each of 58 individuals (Table 1). A standard deviation was calculated for each distribution generated by each run, and average standard deviations for the collinear and noncollinear portions of the genome were calculated from the simulations for those portions of the genome. Markers were judged to have introgressed at rates significantly higher or lower than expected using the standard deviations calculated from the simulations.

RESULTS

Contingency table analysis of marker introgression in the collinear portion of the genome revealed that overall the number of introgressed markers did not differ significantly ($P > 0.05$) from expectations for dominant loci, although there was considerable heterogeneity in the frequency of introgression of individual markers (see below). By contrast, marker introgression in the structurally divergent portion of the genome was significantly lower than would be expected in the absence of resistance to introgression ($P < 0.0001$), as well as significantly lower than in collinear genomic regions ($P < 0.0001$). In fact, no introgression was observed in the rearranged portion of the genome for 33% of the progeny, and the frequency of introgressed markers for the remaining individuals ranged from 0.7% to 2.1%.

The impact of chromosomal structural differences on introgression is graphically illustrated by a composite graphical genotype summarizing information from all 58 progeny (Figure 3). Forty percent of *H. petiolaris* markers in the collinear portion of the genome introgressed in at least one of the 58 progeny, with a total genomic coverage of approximately 40%, whereas only five of 139 (3.6%) *H. petiolaris* markers from the rearranged portion of the genome introgressed, and these covered less than 2.4% of structurally divergent genomic regions. Moreover, four of the five markers

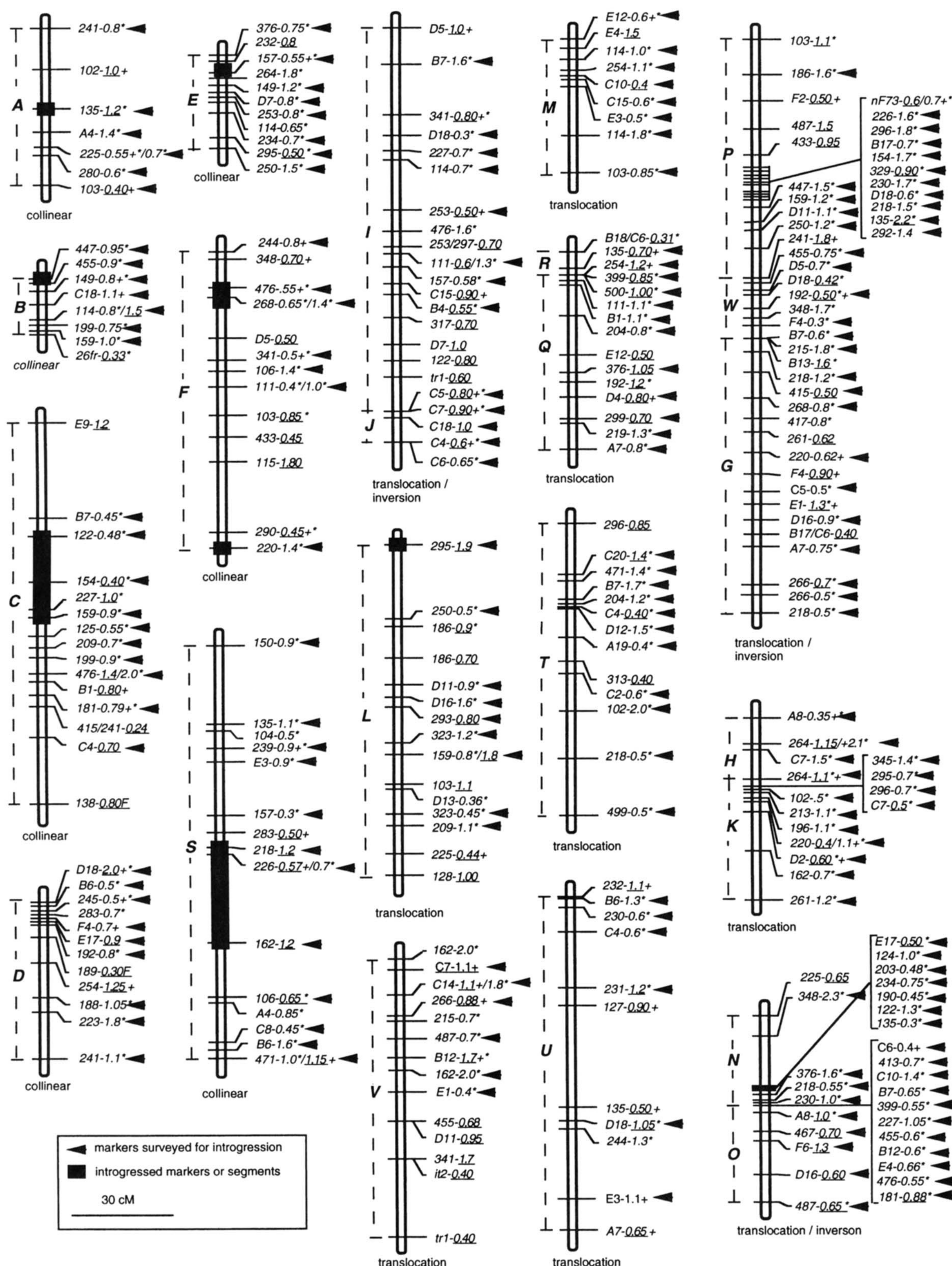

FIGURE 2.—Graphical genotype for one of 58 individuals from a BC_2F_3 progeny of *H. annuus* × *H. petiolaris.* The genomic location of markers from mapping populations of *H. annuus* (underlined), *H. petiolaris* (*), and their hybrid derivative, *H. anomalus* (+), are shown (note that some markers have been mapped in more than one species). Co-dominant loci are indicated by the presence of both "alleles." Markers found in the first generation hybrids, but not in cmsHA89 (the recipient parent), and thus with the potential to introgress, are indicated by arrows. Black bars within linkage groups indicate introgressed markers or putative introgressed chromosomal segments (see MATERIALS AND METHODS). Letters at the left of each linkage group designate major linkage blocks and indicate their relationship to homologous linkages in *H. petiolaris.* Chromosomal structural differences between the species are indicated at the base of each linkage group. Locus nomenclature includes, from left to right, the primer designation and the size in kilobases of the segregating fragment scored.

TABLE 1

Observed and expected proportions of introgressed markers in 0%, 1–25%, 26–50%, and >50% of individuals

Percentage of individuals in which markers introgressed	Entire genome (197 markers)		Collinear portion (58 markers)		Noncollinear portion (139 markers)	
	Observed	Expected	Observed	Expected	Observed	Expected
0%[a]	0.85 (167)	0.0009 (0)	0.57 (33)	0.0006 (0)	0.96 (134)	0.0008 (0)
1–25%	0.07 (13)	0.9662 (190)	0.17 (10)	0.9755 (57)	0.02 (3)	0.9656 (134)
26–50%	0.06 (12)	0.0329 (6.48)	0.17 (10)	0.0239 (1.39)	(0.01) (2)	0.0336 (5)
>50%	0.03 (5)	≪0.0001 (0)	0.09 (5)	≪0.0001 (0)	0.0 (0)	≪0.0001 (0)

[a] Expected values were calculated using the mean standard deviation calculated from the standard deviations of 100 simulations in which there were no barriers to introgression. Values in parentheses are number of markers.

that did introgress were restricted to linkages, that differ between the parental species by simple chromosomal fusions (*L* and *V*, Figure 1), a type of structural rearrangement that theoretically should not inhibit recombination or introgression substantially.

In addition to differences in frequency of introgression observed between collinear and rearranged regions of the genome, significant heterogeneity was observed for rates of introgression within these regions. Comparisons with 100 simulations of unrestricted introgression (Table 1) revealed that the proportion of markers that failed to introgress in both the collinear and rearranged portions of the genome was significantly higher than would be expected by chance (collinear portion: 56.9% observed, $P < 0.0001$; rearranged portion: 96.4% observed, $P < 0.0001$). Likewise, the proportion of markers from the collinear portion of the genome introgressing into >25% of individuals was significantly higher than expected (26% observed, $P < 0.0001$). However, the proportion of markers from structurally divergent genomic regions introgressing into >25% of individuals did not differ significantly from expectations (1.5% observed; $P > 0.5$). Finally, five markers from the collinear portion of the genome introgressed into >50% of the individuals when none were expected to (Table 1).

DISCUSSION

Chromosomal structural differences: The primary observation reported here is that the noncollinear portion of the genome is well-protected from introgression by large translocations and inversions between the parental species, *H. annuus* and *H. petiolaris*. This result is concordant with theory that suggests chromosomal structural differences reduce recombination rates within rearranged linkages and, as a consequence, lower rates of introgression within those regions of the genome (HANSON 1959a,b; GRANT 1981). In some instances, such as the example reported here, the effective reduction in recombination appears to result from selection against recombinant gametes, leading to lower hybrid fertility, whereas in other cases, actual decreases in recombination frequency are observed (SITES and MORITZ 1987; COYNE *et al.* 1993), without loss of fertility. Thus, the efficacy of chromosomal structural differences as reproductive barriers, at least for structurally divergent genomic regions, may not be directly correlated with lowered hybrid fertility.

Because the genomes of the parental species differed by both translocations and inversions, this study initially appeared to provide an opportunity to test the relative effectiveness of these two classes of rearrangements in limiting gene transfer. This was not possible, however, because the inversions are nested within translocated regions, making it difficult to separate the inhibitory effects of the inversions from those of the translocations.

In many groups of plants, introgression appears extensive, yet species differences are maintained (*e.g.*, ARNOLD *et al.* 1991; RIESEBERG *et al.* 1990, 1991; WHITTEMORE and SCHAAL 1991; BRUNSFELD *et al.* 1992; BRUBAKER *et al.* 1993). For example, in the BC_2F_3 progeny array described here, the morphology of the recipient parent, *H. annuus* was completely recovered. No morphological evidence of introgression was observed. One possible explanation for this paradox is that introgression is restricted to a particular set of linkage blocks in these species and that the introgressed regions do not encode species differences. Chromosomal structural differences may provide the selective genomic permeability required by this scenario, at least for structurally divergent taxa. That is, genes affecting species differences may sometimes occur within structurally divergent genomic regions and thus be protected from recombination and subsequent introgression.

Differential introgression for species traits also is possible without chromosomal structural divergence (BARTON and HEWITT 1985; RIESEBERG and ELLSTRAND 1993). Mathematical models indicate that selection against hybrid individuals can create a barrier to introgression for negatively selected loci, as well as closely linked neutral loci (BARTON and GALE 1993). However, advantageous or neutral alleles will be slowed down significantly only if they are tightly linked to loci under negative selection. These models have been validated by observations of

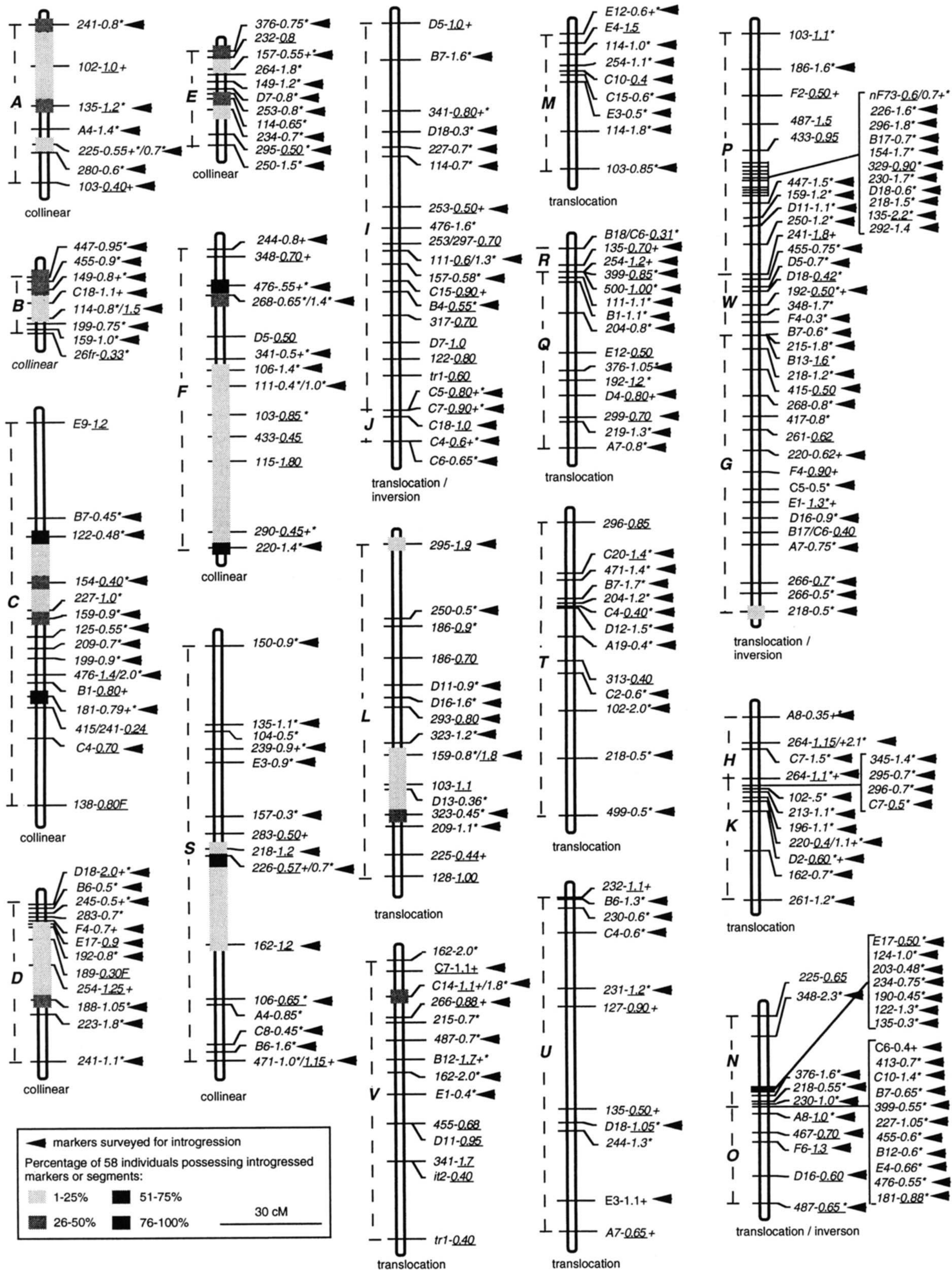

FIGURE 3.—Composite graphical genotype for 58 individuals from a BC_2F_3 progeny of *H. annuus* × *H. petiolaris*. Graphical genotype construction and locus nomenclature follows Figure 2. The percentages of individuals carrying a particular introgressed marker or putative introgressed chromosomal segment are indicated by black or gray bars.

highly variable cline widths for species-specific markers (PARSONS *et al.* 1993; RIESEBERG and ELLSTRAND 1993). Chromosomal structural differences can be considered as one type of negatively selected trait, except that the block of genes protected from introgression will be much larger than for single gene loci.

The low rates of introgression reported here for approximately two thirds of the sunflower genome also have implications for standard plant breeding practices. Clearly, the use of a recurrent backcrossing strategy for the extraction of economically significant traits from structurally divergent genomes is unlikely to be successful if those traits reside in rearranged linkages. A number of theoretical models have suggested that sib-crossing or selfing, in combination with backcrossing, are much more effective than backcrossing alone for introgression across linkage groups where recombination rates are low (HANSON 1959a,b). Experimental studies (HORNER 1968; WALL 1970), appear to confirm these theoretical predictions, but are limited by ambiguity regarding the genetic basis of the features investigated, the chromosomal location of the genes controlling the studied features, and the chromosomal structural differences, if any. Likewise, it is not clear whether the genomic structural resistance to introgression observed in artificial crosses occurs in nature. Experiments currently underway in Helianthus address these questions.

Genic factors: The observation that many markers in the collinear region of the genome introgress at very low rates or not at all suggests that genic factors or small undetected structural changes also may affect rates of introgression in Helianthus. In fact, 60% of the markers from the collinear portion of the genome did not introgress. Meiotic analyses of first generation hybrids between *H. annuus* and *H. petiolaris* (HEISER 1947; CHANDLER *et al.* 1986) indicate bivalent formation between collinear chromosomes and no decrease in crossing over has been reported. Thus, it appears clear that selection against certain *H. petiolaris* genes, combined with linkage, inhibits introgression within the collinear portion of the genome. The effects of genic factors in structurally divergent genomic regions are hard to quantify because they are partially masked by the larger effects of chromosomal rearrangements.

The only other situation where chromosomal and genic components of reproductive barriers are well documented is in the Moreton and Torresion chromosomal races of the grasshopper, *Caledia captiva* (SHAW *et al.* 1986). The two races differ by a series of pericentric inversions involving eight of the 12 chromosomes of the genome. However, because of a chromosomal cline between the two races, SHAW *et al.* (1986) were able to cross populations with substantial genic divergence, but little chromosomal differentiation, as well as populations that were genically equivalent, but differed chromosomally. F_2 inviability improved by 58% in crosses where genic differences were minimized and chromosomal differences were greatest. A 46% increase in viability was observed when the chromosomal differences were removed, but genic differences remained high. Thus, both genic and chromosomal factors contribute to hybrid inviability in *Caledia* (SHAW *et al.* 1986), although their actual impact on introgression between the chromosomal races is unclear. In Helianthus, by way of comparison, the individual contributions of genic and chromosomal factors to hybrid semisterility has not been determined, but both factors clearly reduce levels of interspecific gene flow.

Although selection against chromosomal and genic factors may explain lower than expected levels of introgression, explanations for why certain markers or chromosomal segments introgressed at higher than predicted rates are less apparent. Selection for *H. petiolaris* genes and linkage may account for much of the heterogeneity observed, but alternative explanations involving gene conversion (ARNOLD *et al.* 1988) or recombination hotspots cannot be ruled out. One prediction of the gene conversion hypothesis is that alleles may be introduced into a new genetic background without introducing closely linked markers (HARRISON 1990). This appears to have happened in several instances in Helianthus (Figure 3), providing at least tenuous support for the gene conversion model. For example, in linkage group "*C*", RAPD marker *181-0.79* introgressed at high frequency (Figure 3), but closely linked markers were not found in any of the 58 BC_2F_3 progeny.

Conclusions: Both RAPDs and restriction fragment length polymorphisms (RFLPs) have been used to document the movement of alien genes and/or chromosomal segments across species barriers (*e.g.*, YOUNG and TANKSLEY 1989b; PATERSON *et al.* 1991; ESHED *et al.* 1992; JENA *et al.* 1992; WILLIAMS *et al.* 1993; MCGRATH *et al.* 1994; GARCIA *et al.* 1995). Although these kinds of studies inevitably detect nonrandom patterns of introgression, the factors responsible for these patterns have not been studied in detail.

The results from the introgression experiment presented here provide empirical evidence for at least two mechanisms affecting the frequency and genomic location of introgressed genes in sunflower. Chromosomal structural differences result in differential genomic permeability, with introgression reduced or eliminated in structurally divergent genomic regions. Selection against alien genes has much the same effect, except that resistance to introgression appears to be restricted to smaller genomic regions. Thus, barriers to introgression among sunflower species include both chromosomal structural and genic factors.

We thank M. DEBACON for technical assistance, and R. NOYES, and J. WHITTON for helpful discussions and recommendations to improve the manuscript. This research was supported by National Science Foundation grant BSR-9419206 and U.S. Department of Agriculture grant 92373007590 to L.H.R.

LITERATURE CITED

ABBOTT, R. J., 1992 Plant invasions, interspecific hybridization and the evolution of new plant taxa. TREE **7:** 401-404.
ANDERSON, E., 1949 *Introgressive Hybridization.* John Wiley, New York.
ARNOLD, M. L., 1992 Natural hybridization as an evolutionary process. Annu. Rev. Ecol. Syst. **23:** 237-261.

ARNOLD, M. L., 1994 Natural hybridization and Louisiana irises. BioSciences **44:** 141–147.

ARNOLD, M. L., D. D. SHAW and N. CONTRERAS, 1987 Ribosomal RNA encoding DNA introgression across a narrow hybrid zone between two subspecies of grasshopper. Proc. Natl. Acad. Sci. USA **84:** 3946–3950.

ARNOLD, M. L., N. CONTRERAS and D. D. SHAW, 1988 Biased gene conversion and asymmetrical introgression between subspecies. Chromosoma **96:** 368–371.

ARNOLD, M. L., B. D. BENNETT and E. A. ZIMMER, 1990 Natural hybridization between *I. fulva* and *I. hexagona*: patterns of ribosomal DNA variation. Evolution **44:** 1512–1521.

ARNOLD, M. L., C. M. BUCKNER and J. J. ROBINSON, 1991 Pollen mediated introgression and hybrid speciation in Louisiana irises. Proc. Natl. Acad. Sci. USA **88:** 1398–1402.

BARBER, H. N., and W. D. JACKSON, 1957 Natural selection in action in *Eucalyptus*. Nature **179:** 1267–1269.

BARTON, N. H., and K. S. GALE, 1993 Genetic analysis of hybrid zones, pp. 13–45 in *Hybrid Zones and the Evolutionary Process*, edited by R. G. HARRISON. Oxford University Press, New York.

BARTON, N. H., and G. M. HEWITT, 1985 Analysis of hybrid zones. Annu. Rev. Ecol. Syst. **16:** 113–148.

BRUBAKER, C. L., J. A. KOONTZ and J. F. WENDEL, 1993 Bidirectional cytoplasmic and nuclear introgression in the New World cottons, *Gossypium barbadense* and *G. hirsutum* (Malvaceae). Am. J. Bot. **80:** 1203–1208.

BRUNSFELD, S. B., D. E. SOLTIS and P. S. SOLTIS, 1992 Evolutionary patterns and processes in *Salix* sect. *Longifoliae*: evidence from chloroplast DNA. Syst. Bot. **17:** 239–256.

CHANDLER, J. M., C. JAN and B. H. BEARD, 1986 Chromosomal differentiation among the annual *Helianthus* species. Syst. Bot. **11:** 353–371.

COYNE, J. A., W. MEYERS, A. P. CRITTENDEN and P. SNIEGOWSKI, 1993 The fertility effects of pericentric inversions in *Drosophila melanogaster*. Genetics **134:** 487–496.

DOEBLEY, J. F., 1989 Molecular evidence for a missing wild relative of maize and the introgression of its chloroplast genome into *Zea perennis*. Evolution **43:** 1555–1558.

DORADO, O., L. H. RIESEBERG and D. ARIAS, 1992 Chloroplast DNA introgression in southern California sunflowers. Evolution **46:** 566–572.

DOYLE, J. J., and J. L. DOYLE, 1987 A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. **19:** 11–15.

ESHED, Y., M. ABU-ABIED, Y. SARANGA and D. ZAMIR, 1992 *Lycopersicon esculentum* lines containing small overlapping introgressions from *Lycopersicon pennellii*. Theor. Appl. Genet. **83:** 1027–1034.

GARCIA, G. M., H. T. STALKER and G. KOCHERT, 1995 Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers. Genome **38:** 166–176.

GOTTLIEB, L. D., 1972 Levels of confidence in the analysis of hybridization in plants. Ann. Missouri Bot. Gard. **59:** 435–446.

GRANT, V., 1981 *Plant Speciation*. Columbia University Press, New York.

HANSON, W. D., 1959a Early generation analysis of lengths of chromosome segments around a locus held heterozygous with backcrossing or selfing. Genetics **44:** 833–837.

HANSON, W. D., 1959b The breakup of initial linkage blocks under selected mating systems. Genetics **44:** 857–868.

HARDIN, J. W., 1975 Hybridization and introgression in *Quercus alba*. J. Arnold Abor. **56:** 336–363.

HARRISON, R. G., 1990 Hybrid zones: windows on evolutionary processes. Oxford Surv. Evol. Biol. **7:** 69–128.

HEISER, C. B., 1947 Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. Evolution **1:** 249–262.

HEISER, C. B., 1973 Introgression re-examined. Bot. Rev. **39:** 347–366.

HORNER, E. S., 1968 Effect of a generation of inbreeding on genetic variation in corn (*Zea mays* L.) as related to recurrent selection procedures. Crop. Sci. **8:** 32–35.

JACKSON, R. C., 1985 Genomic differentiation and its effect on gene flow. Syst. Bot. **10:** 391–404.

JENA, K. K., G. S. KHUSH and G. KOCHERT, 1992 RFLP analysis of rice (*Oryza sativa* L.) introgression lines. Theor. Appl. Genet. **84:** 608–616.

KEIM, P., K. N. PAIGE, T. G. WHITHAM and K. G. LARK, 1989 Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. Genetics **123:** 557–565.

MCGRATH, J. M., S. M. WIELGUS, T. G. UCHYTIL, H. KIM-LEE, G. T. HABERLACH *et al.*, 1994 Recombination of *Solanum brevidens* chromosomes in the second backcross generation from a somatic hybrid with *S. tuberosum*. Theor. Appl. Genet. **88:** 917–924.

PARSONS, T. J., S. L. OLSON and M. J. BRAUN, 1993 Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. Science **260:** 1643–1646.

PATERSON, A. H., S. DAMON, J. D. HEWITT, D. ZAMIR, H. D. RABINOWITCH *et al.*, 1991 Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics **127:** 181–197.

PATTON, J. L., 1993 Hybridization and hybrid zones in pocket gophers, pp. 290–308 in *Hybrid Zones and the Evolutionary Process*, edited by R. G. HARRISON. Oxford University Press, New York.

RANDOLPH, L. G., I. S. NELSON and R. L. PLAISTED, 1967 Negative evidence of introgression affecting the stability of Louisiana Iris species. Cornell Univ. Agr. Expt. Stat. Mem. **398:** 1–56.

RAVEN, P. H., 1976 Systematics and plant population biology. Syst. Bot. **1:** 284–316.

RIESEBERG, L. H., 1995a Homology among RAPD fragments in interspecific comparisons. Mol Ecol (in press).

RIESEBERG, L. H., 1995b Invited Special Paper. The role of hybridization in evolution: Old wine in new skins. Am. J. Bot. **82:** 944–953.

RIESEBERG, L. H., and S. J. BRUNSFIELD, 1992 Molecular evidence and plant introgression, pp. 151–176 in *Molecular Systematics of Plants*, edited by D. E. SOLTIS, P. S. SOLTIS and J. J. DOYLE. Chapman and Hall Inc., New York.

RIESEBERG, L. H., and N. C. ELLSTRAND, 1993 What can morphological and molecular markers tell us about plant hybridization. Crit. Rev. Plant Sci. **12:** 213–241.

RIESEBERG, L. H., and D. E. SOLTIS, 1991 Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Plants **5:** 65–84.

RIESEBERG, L. H., and J. WENDEL, 1993 Introgression and its consequences in plants, pp. 70–109 in *Hybrid Zones and the Evolutionary Process*, edited by R. G. HARRISON. Oxford University Press, New York.

RIESEBERG, L. H., S. BECKSTROM-STERNBERG and K. DOAN, 1990 *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. Proc. Natl. Acad. Sci. USA **87:** 593–597.

RIESEBERG, L. H., H. CHOI and D. HAM, 1991 Differential cytoplasmic versus nuclear gene flow in *Helianthus*. J. Hered. **82:** 489–493.

RIESEBERG, L. H., C. VAN FOSSEN and A. DESROCHERS, 1995 Genomic reorganization accompanies hybrid speciation in wild sunflowers. Nature **375:** 313–316.

SEARLE, J. B., 1993 Chromosomal hybrid zones in eutherian mammals, pp. 309–354 in *Hybrid Zones and the Evolutionary Process*, edited by R. G. HARRISON. Oxford University Press, New York.

SEILER, G. J., 1991 Registration of 15 interspecific sunflower germplasm lines derived from wild annual species. Crop Sci. **31:** 1389–1390.

SHAW, D. D., D. J. COATES and P. WILKINSON, 1986 Estimating the genic and chromosomal components of reproductive isolation within and between subspecies of the grasshopper *Caledia captiva*. Can. J. Genet. Cytol. **28:** 686–695.

SHAW, D. D., A. D. MARCHANT, N. CONTRERAS, M. L. ARNOLD, F. GROETERS *et al.*, 1993 Genomic and environmental determinants of a narrow hybrid zone, pp. 165–195 in *Hybrid Zones and the Evolutionary Process*, edited by R. G. HARRISON. Oxford University Press, New York.

SITES, J. W., and C. MORITZ, 1987 Chromosomal evolution and speciation revisited. Systematic Zoology **36:** 153–174.

SMITH, R. L., and K. J. SYTSMA, 1990 Evolution of *Populus nigra* L. (sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* L. (sect. *Populus*). Am. J. Bot. **77:** 1176–1187.

STEBBINS, G. L., 1950 *Variation and Evolution in Plants*. Columbia University Press, New York.

STEBBINS, G. L., 1971 *Chromosomal evolution in higher plants*. Addison-Wesley, Reading, MA.

SYBENGA, J. 1992 *Cytogenetics in Plant Breeding. Monographs on Theoretical and Applied Genetics.* Springer-Verlag, Berlin.

WAGNER, W. H., JR., 1969 The role and taxonomic treatment of hybrids. BioScience **19:** 785–789.

WAGNER, W. H., JR., 1970 Biosystematics and evolutionary noise. Taxon **19:** 146–151.

WALL, J. R., 1970 Experimental introgression in the genus *Phaseolus.* I. Effect of mating systems on interspecific gene flow. Evolution **24:** 356–366.

WHITE, M. J. D., 1978 *Modes of Speciation.* W. H. Freeman and Company, San Francisco.

WHITKUS, R., J. DOEBLEY and M. LEE, 1992 Comparative genome mapping of sorghum and maize. Genetics **132:** 1119–1130.

WHITTEMORE, A. T., and B. A. SCHAAL, 1991 Interspecific gene flow in oaks. Proc. Natl. Acad. Sci. USA **88:** 2540–2544.

WILLIAMS, J. G. K., A. R. KUBELIK, K. J. LIVAK, J. A. RAFALSKY and S. V. TINGEY, 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:** 6531–6535.

WILLIAMS, C. E., S. M. WIELGUS, G. T. HARBERLACH, C. GUENTHER, H. KIM-LEE *et al.*, 1993 RFLP analysis of chromosomal segregation in progeny from an interspecific hexaploid hybrid between *Solanum brevidens* and *Solanum tuberosum.* Genetics **135:** 1167–1173.

YOUNG, N. D., and S. D. TANKSLEY, 1989a Restriction fragment length polymorphism maps and the concept of graphical genotypes. Genetics **77:** 95–101.

YOUNG, N. D., and S. D. TANKSLEY, 1989b RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. Theor. Appl. Genet. **77:** 353–359.

Communicating editor: J. A. BIRCHLER