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L. H. Rieseberg · D. M. Arias · M. C. Ungerer
C. R. Linder · B. Sinervo

The effects of mating design on introgression between chromosomally divergent sunflower species

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Abstract Population genetic theory suggests that mating designs employing one or more generations of sib-crossing or selfing prior to backcrossing are more effective than backcrossing alone for moving alleles across linkage groups where effective recombination rates are low (e.g., chromosomally divergent linkages). To test this hypothesis, we analyzed the effects of chromosomal structural differences and mating designs on the frequency and genomic distribution of introgressed markers using the domesticated sunflower, *Helianthus annuus*, and one of its wild relatives, *H. petiolaris*, as the experimental system. We surveyed 170 progeny, representing the end products of three different mating designs (design I, P-F_1-BC_1-BC_2-F_2- F_3; design II, P-F_1-F_2-BC_1-BC_2-F_3; and design III, P-F_1-F_2-F_3-BC_1-BC_2), for 197 parental RAPD markers of known genomic location. Comparison of observed patterns of introgression with expectations based on simulations of unrestricted introgression revealed that much of the genome was protected from introgression regardless of mating design or chromosomal structural differences. Although the simulations indicated that all markers should introgress into multiple individuals in each of the three mating designs, 20 of 58 (34%) markers from collinear linkage groups, and 112 of 139 (81%) markers from rearranged linkage groups did not introgress. In addition, the average size of introgressed fragments (12.2 cM) was less than half that predicted by theoretical models (26–33 cM). Both of these observations are consistent with strong selection against introgressed linkage blocks, particularly in chromosomally divergent linkages. Nonetheless, mating designs II and III, which employed one and two generations of sib-mating, respectively, prior to backcrossing, were significantly more effective at moving alleles across both collinear and rearranged linkages than mating design I, in which the backcross generations preceded sib-mating. Thus, breeding strategies that include sib-crossing, in combination with backcrossing, should significantly increase the effectiveness of gene transfer across complex genic or chromosomal sterility barriers.

Key words Sunflower · *H. annuus* · Random amplified polymorphic DNA (RAPD) · Introgression · Plant breeding

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L. H. Rieseberg (✉) · M. Ungerer · C. R. Linder · B. Sinervo
Department of Biology, Indiana University, Bloomington, IN 47405, USA

D. M. Arias
Centro de Investigación Ambiental E Investigación Sierra de Huautla, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico 62210

Introduction

Interspecific gene transfer through sexual hybridization and introgression continues to be the predominant method for introducing new alleles into many domesticated plant species. Nonetheless, there are two major difficulties with this approach. First, natural populations with the desired trait often will not hybridize with the domesticated plant due to genic or chromosomal incompatibility (Gasser and Fraley 1989). Second, the features of interest may be in the sexual breeding pool, but inaccessible to conventional breeding techniques because they are under multigene control (Tanksley et al. 1989) and/or because they are linked to genes or chromosomal rearrangements that contribute to reduced hybrid viability or fertility (i.e., hybrid fitness). The first problem has attracted a great deal of interest in the plant genetic and breeding community, ultimately leading to the development of genetic engineering technologies which allow the transfer of genes through non-sexual means (Gasser and Fraley 1989; Gotsch and Rieder 1989; Ratner 1989). Likewise, the development of genetic linkage maps has facilitated the breeding of quantitative traits (Tanksley et al. 1989). The focus of the present paper, however, concerns traits present in the breeding pool, but which are often inaccessible due to linkage with genic and/or chromosomal factors contributing to reduced hybrid fitness.

The permeability of genetically differentiated genomes to introgression will largely depend on the number, genomic location, and interactions of genetic factors contributing to reduced hybrid fertility or viability (Barton and Hewitt 1985). To move alleles across a species barrier, an allele must recombine into a new genetic background before it is eliminated by selection against the alleles or chromosomal rearrangements with which it is initially associated (Barton and Hewitt 1985). If many genes or chromosomal rearrangements contribute to reduced hybrid fitness, then much of the genome may be resistant to introgression due to linkage (Whittemore and Schaal 1991; Rieseberg and Wendel 1993), particularly if recombination rates are low. This problem may be exacerbated if the genes interact epistatically or are "co-adapted" (Harlan 1936; Carson 1975). On the other hand, if these reproductive barriers are under simple genetic control, then most of the genome should be permeable to introgression. Only those traits tightly linked to sterility genes or chromosomal rearrangements will be difficult to introgress. Thus, the genetic architecture of reproductive barriers has predictable consequences for the introgression of traits.

Theory suggests that the movement of alleles across reproductive barriers can be enhanced by increasing recombination rates between parental linkage blocks (Hanson 1959a, b; Stephens 1961; Wall 1970). Unfortunately, recurrent backcrossing, the mating design typically employed for breeding purposes, has been demonstrated theoretically to be an extremely inefficient mechanism for the break-up of parental linkage blocks (Hanson 1959a, b) because recombination between the parental linkage blocks is not promoted. This problem is exacerbated for chromosomes with a short map length, where the disruption of parental linkage blocks will be even slower due to lower recombination rates (Hanson 1959a). Hanson (1959b) also noted that chromosomal structural differences have the effect of greatly reducing recombination rates and subsequent map lengths. Thus, he recommended mating designs that enhance recombination between the parental genomes be employed in these types of situations to ensure the disruption of parental linkage groups. Selfing and sib-mating represent two such systems (e.g., Liu et al. 1996).

A second requirement for the successful introgression of alleles is the maintenance of reasonable levels of fertility and viability in hybrid or backcross populations. Unfortunately, populations resulting from either selfing or sib-mating will generally exhibit lower levels of fertility than those resulting from recurrent backcrossing (Wall 1970). In particular, selfing tends to result in high proportions of "subvital" plants due to disharmonious genic or chromosomal combinations, a phenomenon termed hybrid breakdown (Stebbins 1950; Stephens 1950). Given these considerations, Wall (1970) suggested that there is an optimal level of recombination between genetically or chromosomally divergent populations. If recombination is too low, no introgression occurs, whereas if it is too high it may severely reduce overall viability and fertility in the resulting population. Thus, Wall (1970) argued that mating designs employing one or more generations of sib-mating interspersed with backcrossing will be more effective than backcrossing alone for moving alleles across linkage groups where effective recombination rates are low, such as in chromosomally divergent linkages. Likewise, Hanson (1959b) suggested that as many as four sib-generations may be necessary to break-up parental linkage groups in breeding experiments involving chromosomally divergent parental populations.

In this paper, we compare the performance of three different mating designs for moving alleles between two karyotypically divergent sunflower species, *Helianthus petiolaris* and *H. annuus*. All three designs included two generations of sib-mating and backcrossing, but differed in the order of the backcross and sib-cross generations. We predicted that mating designs in which sib-mating preceded backcrossing would be more effective at moving alleles across the species barrier than mating designs in which the backcross generations precede sib-mating. Furthermore, these differences were predicted to be most pronounced in chromosomally divergent linkages where effective recombination rates are low.

Materials and methods

Plant materials

H. annuus and *H. petiolaris*are self-incompatible annual sunflower species with the same chromosome number (n=17). Both species are native to North America and are abundant in the western United States. *H. annuus* also includes the domesticated sunflower which is self-compatible. Comparative genetic linkage mapping (Rieseberg et al. 1995b) indicates that seven linkage groups are collinear between the two species, whereas the remaining ten linkages differ structurally due to a minimum of seven interchromosomal translocations and three inversions. These structural changes generate multivalent formation 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, fertility is rapidly restored in later generation hybrids and backcrosses (Heiser 1947). In addition to the chromosomal rearrangements, patterns of introgression indicate that genic factors contribute to reproductive isolation as well (Rieseberg et al. 1995a).

To analyze the effects of mating design on the genomic location and rate of introgression, three introgression lines were generated between *H. annuus* and *H. petiolaris*: mating design I, P-F_1-BC_1-BC_2-F_2- F_3; mating design II, P-F_1-F_2-BC_1-BC_2-F_3; and mating design III, P-F_1-F_2-F_3-BC_1-BC_2. The initial interspecific cross was *H. annuus* (cmsHA89, female) × *H. petiolaris* subsp. *petiolaris* (PET-PET-1741-1, male Seiler 1991). Backcrosses were in the direction of *H. annuus*, the maternal parent. Due to self-incompatibility, sib-mating was employed rather than selfing for the F-generations. At least 20 plants were used for each generation. Crosses were performed by applying pooled pollen from all plants from a given generation to stigmas of the same individuals. All achenes from each generation were pooled, and 30 or more achenes were arbitrarily chosen as founders of the next generation. For each mating design, 56–58 end products were grown to a size sufficient for total DNA isolation.

DNA isolation

DNAs were isolated and purified as described by Rieseberg et al. (1995a). Briefly, fresh leaf tissue was ground in a CTAB extraction buffer (Whitkus et al. 1992), 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, Inc.) and then quantified on a fluorometer.

RAPD marker surveys

The 170 purified progeny DNAs were surveyed for the 197 mapped random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) described in Rieseberg et al. (1995a, b). 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 80% (about 1160 cM) of the sunflower genome currently mapped (Berry et al. 1995; Gentzbittel et al. 1995; Rieseberg et al. 1995b;), with an average distance of 6.5 cM between markers based on *H. annuus* map distances (Rieseberg et al. 1995a, b).

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 of primer (15 ng), and a final concentration of 2 mM $MgCl_2$, 20 mM Tris-HCl, 100 µM of each dNTP, and 1 U of *Taq* DNA polymerase. The reactions were overlaid with mineral oil and placed in an MJ Research Thermal Cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C followed by a final extension at 72°C for 7 min. Amplification products were separated by electrophoresis in 1.5% TBE agarose gels and detected by staining with ethidium bromide.

Graphical genotype construction

Graphical genotype construction is described in detail in Rieseberg et al. (1995a). Briefly, 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 170 progeny. 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 were often 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. Nonetheless, because the mating designs included two backcross generations, and because of the low frequency of many introgressed markers, it is likely that most introgressed markers are heterozygous.

Data analyses

To determine whether the overall, collinear, and non-collinear patterns of introgression observed in the three mating designs differed from what would be expected if there were no barriers to introgression, we performed simulations of unrestricted introgression for each of the three views of the genome as described in Rieseberg et al. (1995a).

Lengths of introgressed fragments were estimated from prior mapping of the RAPD markers (Rieseberg et al. 1995b) and followed the general guidelines of Wang et al. (1995). Briefly, the length of a fragment was estimated as the distance spanned by all consecutive introgressed markers, plus half the distance between the terminal introgressed markers and the nearest non-introgressed markers. Introgressed fragment lengths from incomplete genotypes were estimated in a manner that minimized their size. That is, markers that could not be scored were assumed to be absent, rather than present, for the purposes of estimating fragment lengths. Finally, as mentioned above, it was assumed that adjacent introgressed markers were in coupling phase and heterozygous.

The effects of mating design on the total number of markers introgressed for the entire genome, as well as for the collinear and non-collinear regions, were tested by contingency table analysis (Sokal and Rohlf 1995). One-way analysis of variance (ANOVA) was used to test the effect of mating design on the total length of introgressed fragments per individual. The effect of mating design on natural-log transformed introgressed fragment lengths for the entire genome, for collinear and non-collinear genomic regions, and for each chromosome, were tested by two-way, two-way, and one-way ANOVA, respectively. Normality was checked by plotting residuals on probability plots. Linear regressions were performed to test the effect of chromosome map length on introgressed fragment length in both the collinear and rearranged portions of the genome. The regressions were conducted for the entire genome, as well as for each mating design. We also tested for differences in slope between collinear and rearranged genomic regions using one-way ANOVA with ln(chromosome length) as a covariate. For all analyses involving multiple *post hoc* tests of significance, experiment-wise error rate (α=0.05) was controlled using the sequential Bonferroni method (Rice 1989).

Results

Frequency of marker introgression

The expected frequency of introgression for dominant markers based on 100 simulations of unrestricted marker introgression was 0.156±0.0504 for mating design I, 0.1878±0.0515 for mating design II, and 0.2487±0.0578 for mating design III (Table 1). However, actual frequencies of marker introgression were much lower. For mating design I, 167 of 197 (85%) *H. petiolaris* markers did not introgress, whereas all were expected to assuming unrestricted introgression. Likewise, all 197 markers were expected to introgress in mating design II, but 150 of 197 (76%) did not. A similar pattern was observed for mating design III, in which 140 of 197 (71%) *H. petiolaris* markers did not introgress when all were expected to assuming no barriers to introgression.

As was previously reported for mating design I (Rieseberg et al. 1995a), the genomic distribution of introgressed markers or linkage blocks is strongly correlated with the location of chromosomal structural differences between *H. annuus* and *H. petiolaris*. Much lower frequencies of introgression were observed in the rearranged than in the collinear portion of the genome for all three mating designs (Tables 1, 2; Fig. 1). The most striking result is from mating design I, where 26 of 58 (45%) *H. petiolaris* markers in the collinear portion of the genome introgressed in at least 1 of the 56 progeny, with a total genomic coverage of approximately 54% (Fig. 1; Tables 1, 2). By contrast, only 5 of 139 (3.6%) *H. petiolaris* markers from the rearranged portion of the genome introgressed, and these covered less than 3.8% of structurally divergent genomic regions. In mating design II, 35 of 58 (60%) of *H. petiolaris* markers from collinear linkages introgressed in at least one of the 56 progeny, whereas only 12 of 139 (8.6%) *H. petiolaris* markers from the rearranged portion of the genome introgressed (Fig. 1; Tables 1, 2). Likewise, in mating design

Table 1 Observed and expected proportions of markers introgressed into 0%, 1–25%, 26–50%, and >50% of individuals based on 100 simulations of unrestricted marker introgression for the entire genome, as well as within the collinear and rearranged portions of the genome

Percentage of individuals in which markers introgressed	Entire genome (179 markers)		Collinear portion (58 markers)		Rearranged portion (139 markers)	
	Observed	Expected[a]	Observed	Expected	Observed	Expected
Mating design I:						
0%	0.85	0.0009	0.57	0.0006	0.96	0.0008
1–25%	0.07	0.9662	0.17	0.9755	0.01	0.9656
26–50%	0.06	0.0329	0.17	0.0239	0.01	0.0336
>50%	0.03	<0.0001	0.09	<0.0001	0.0	<0.0001
Mating desgin II:						
0%	0.76	0.0001	0.40	0.0003	0.91	0.0003
1–25%	0.08	0.8868	0.19	0.8596	0.03	0.8746
26–50%	0.12	0.1131	0.24	0.1401	0.06	0.1251
>50%	0.05	<0.0001	0.17	<0.0001	0.0	<0.0001
Mating design III:						
0%	0.71	<0.0001	0.38	<0.0001	0.85	<0.0001
1–25%	0.17	0.5080	0.32	0.4443	0.11	0.4880
26–50%	0.05	0.4920	0.12	0.5557	0.02	0.5120
>50%	0.07	<0.0001	0.17	<0.0001	0.02	<0.0001

[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. Mating design I: 0.156±0.0429 (μ±σ); mating design II: 0.1878±0.0515; mating design III: 0.2487±0.0578

Table 2 Chi-square tests of the effects of mating design on the number of markers introgressed over all individuals for the entire, collinear, and rearranged portions of the genome. Total genomic coverage by the introgressed markers is given in parentheses. Significant differences were tested using the sequential Bonferroni method (experimentwise error rate $\alpha=0.05$) and are indicated by an asterisk

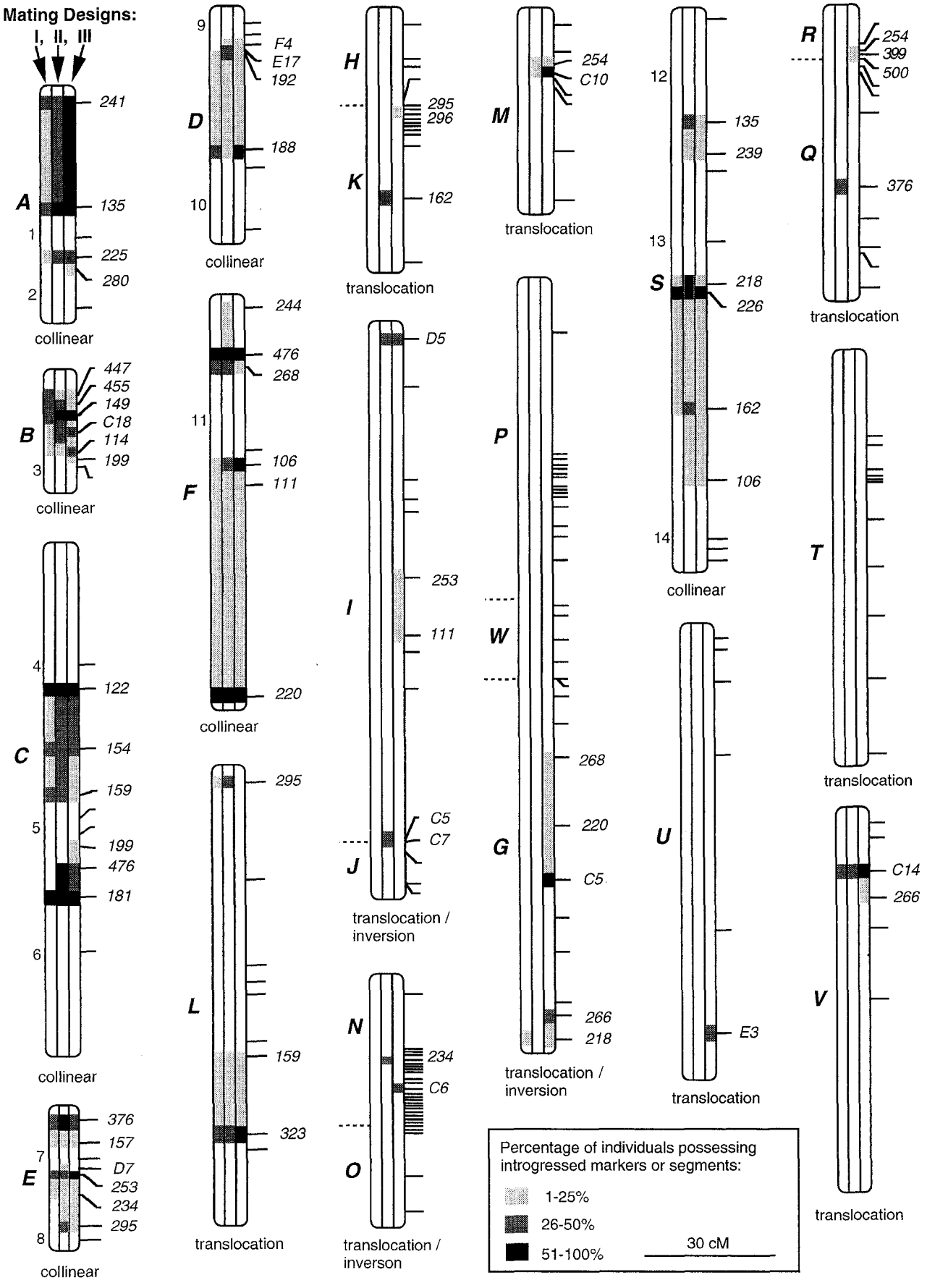
Mating design	Number of markers introgressed		
	Overall	Collinear linkages	Rearranged linkages
I	31 (242 cM)[a]	26 (212 cM)	5 (30 cM)
II	47 (379 cM)	34 (299 cM)	13 (80 cM)
III	57 (404 cM)	36 (300 cM)	21 (104 cM)
Chi-square tests	χ^2	P	
Overall:			
I × II	4.6	<0.05	
I × III	9.9	<0.001*	
II × III	1.1	NS	
Collinear:			
I × II	2.8	NS	
I × III	3.5	NS	
II × III	0.15	NS	
Rearranged:			
I × II	3.8	0.05	
I × III	10.9	<0.001*	
II × III	2.1	NS	

[a] Estimates of genomic coverage for mating design I are higher than previously reported (Rieseberg et al. 1995a) because minimum fragment sizes were used in the earlier estimates

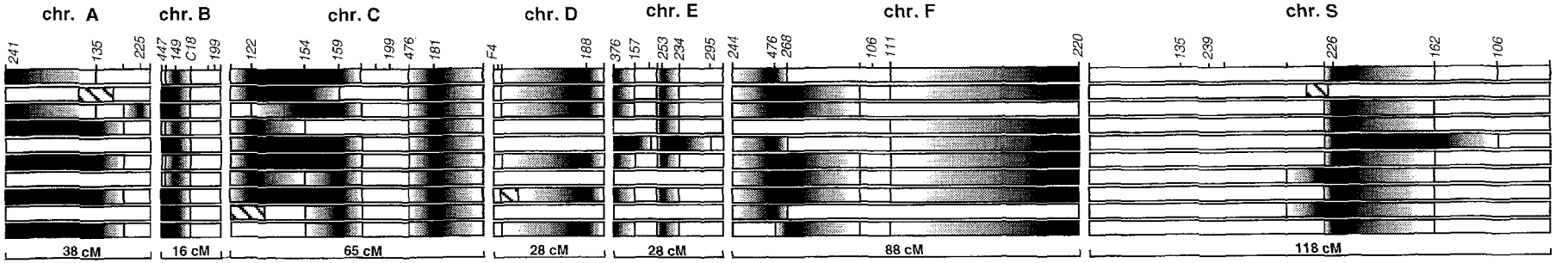
III, 36 of 58 (62%) of *H. petiolaris* markers in the collinear portion of the genome introgressed in at least one of the 56 progeny, compared to only 21 of 139 (15%) *H. petiolaris* markers from the rearranged portion of the genome. Contingency table analysis revealed that these differences were highly significant for each of the three mating designs ($P \ll 0.0001$).

In addition to differences in rates of introgression between the collinear and rearranged regions of the genome, significant heterogeneity was observed for rates of introgression within these genomic regions as well (Figs. 1, 2; Table 1). For example, 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 for all three mating designs (Table 1; $P \ll 0.0001$ for all genomic regions and all mating designs). Likewise, the proportion of markers from the collinear portion of the genome introgressing into >25% of individuals was significantly higher than expected for mating designs I and II ($P \ll 0.0001$ for both), but significantly

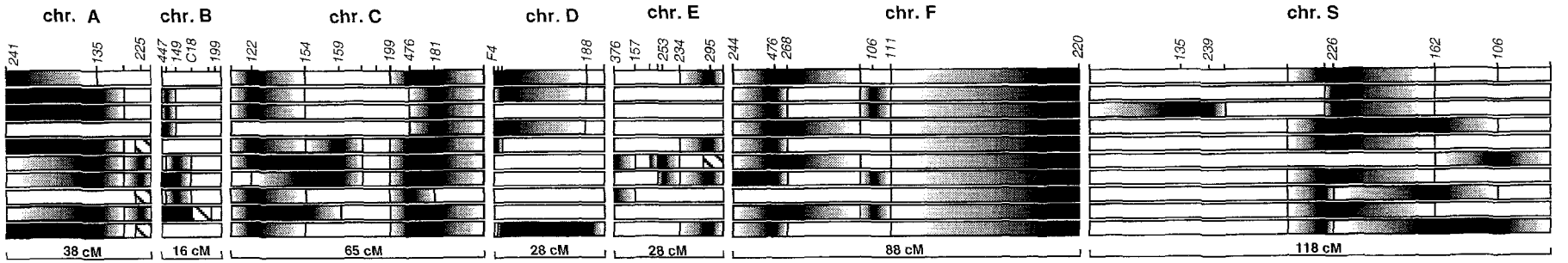
Fig. 1 Composite graphical genotypes of three introgression lines generated between *H. annuus* and *H. petiolaris*: mating design I, P-F_1-BC_1-BC_2-F_2- F_3; mating design II, P-F_1-F_2-BC_1-BC_2-F_3; and mating design III, P-F_1-F_2-F_3-BC_1-BC_2. Graphical genotypes for mating designs I, II, and III are in the left, center, and right side, respectively, of each linkage group. The graphical genotypes are based on the 1084-cM map of *H. annuus* extended here by approximately 290 cM because several *H. petiolaris* markers occur outside currently mapped regions in *H. annuus* (Rieseberg et al. 1995b). *Letters* at the left of each linkage group designate major linkage blocks and indicate their relationship to homologous linkages in *H. petiolaris* (Rieseberg et al. 1995b). *Horizontal lines* extending to the right of linkage groups indicate the genomic location of the 197 *H. petiolaris* RAPD markers surveyed (Rieseberg et al. 1995a); introgressed markers are identified by *primer numbers*. *Black or gray bars* indicate the frequency of introgressed *H. petiolaris* markers. *Numbers* at the left of linkage groups indicate linkage blocks that resist introgression in all three mating designs and thus probably contribute to hybrid unfitness. Because of dominance, we were often unable to determine whether the *H. petiolaris* markers or segments were present in the homozygous or heterozygous condition


Mating Designs:
I, II, III
241
A
135
1
225
280
2
collinear
447
455
149
C18
B
114
199
3
collinear
4
122
C
154
159
5
199
476
181
6
collinear
376
157
7
D7
E
253
234
295
8
collinear
9
F4
E17
192
D
188
10
collinear
244
476
268
11
106
F
111
220
collinear
295
L
159
323
translocation
H
295
296
K
162
translocation
D5
253
I
111
C5
C7
J
translocation /
inversion
N
234
C6
O
translocation /
inverson
254
C10
M
translocation
P
W
268
220
G
C5
266
218
translocation /
inversion
12
135
239
13
S
218
226
162
106
14
collinear
E3
U
translocation
R
254
399
500
Q
376
translocation
T
translocation
C14
266
V
translocation
Percentage of individuals possessing
introgressed markers or segments:
1-25%
26-50%
51-100%
30 cM

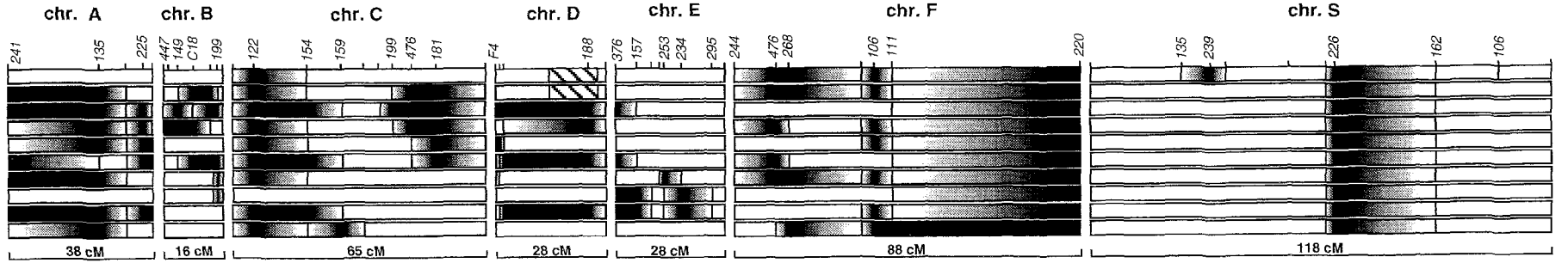
Mating Design I:



Mating Design II:



Mating Design III:



H. annuus linkage blocks; *H. petiolaris* linkage blocks; uncertain due to missing data

lower than expected for mating design III ($P<0.01$). By contrast, the proportion of markers from structurally divergent genomic regions introgressing into >25% of individuals did not differ significantly from expectations for mating design I ($P>0.5$), but was significantly lower than expectations for mating designs II ($P<0.05$) and III ($P\ll0.0001$). Finally, five, ten, and ten markers introgressed into greater than 50% of individuals in the collinear portion of the genome for mating designs I, II, and III, respectively, when none were expected to. In the rearranged portion of the genome, as predicted by the simulations, no markers introgressed at a frequency of greater than 50% in mating designs I and II. However, three markers introgressed into greater than 50% of individuals in mating design III.

Mating design had a significant impact on the overall number and genomic distribution of introgressed markers (Table 2). Of the 197 markers assayed, 31 (15.7%), 47 (23.9%), and 57 (28.9%) introgressed in mating designs I, II, and III, respectively. These differences were significant only for comparisons of mating design I with mating design III (Table 2). A similar pattern was observed when the comparisons were restricted to the rearranged portion of the genome: mating design I differed significantly from mating design III in terms of introgressed marker frequency, but no significant differences were observed for other comparisons. By contrast, no significant differences in the number of introgressed markers were observed for comparisons among the three mating designs for the collinear portion of the genome (Table 2).

Lengths of introgressed linkage blocks

No significant differences were observed among mating designs for ln-transformed fragment lengths (Table 3). However, fragment lengths were significantly longer for collinear than rearranged linkages, and there was significant interaction between mating design and genomic region (Table 3; Fig. 3). A modest increase in fragment length was observed for mating designs II and III relative to mating design I for collinear linkages, whereas the trend was reversed for rearranged linkages (Fig. 3). In all instances, however, mean fragment lengths from collinear linkages were greater than those from the rearranged portion of the genome.

Comparison of ln-transformed fragment lengths among the three mating designs on a chromosome by chromosome basis also revealed few significant differences (Table 4). Two comparisons were significant at an experiment-wise error rate of $\alpha=0.05$: linkage group HK (mating designs II III); and linkage group PWG (mating designs I III), but these differences may be an artifact of the infrequent introgression observed across these linkage groups (Table 4).

As predicted by Hanson (1959a), chromosome map length did affect introgressed fragment lengths ($x^2=156.6$; $P\ll0.0001$), with longer fragments occurring on longer chromosomes (Fig. 4). With the exception of rearranged linkages from mating design I, this relationship was observed for both collinear and rearranged genomic regions and for all three mating designs (Fig. 4). Too few markers (five) introgressed into rearranged linkages in mating design I to get an accurate picture of how fragment length is affected by chromosome length in this instance. Within the range of the data, rearranged fragment sizes were consistently smaller than collinear fragments, but in mating design III rearranged fragments increased in size more rapidly than collinear fragments as chromosome length in-

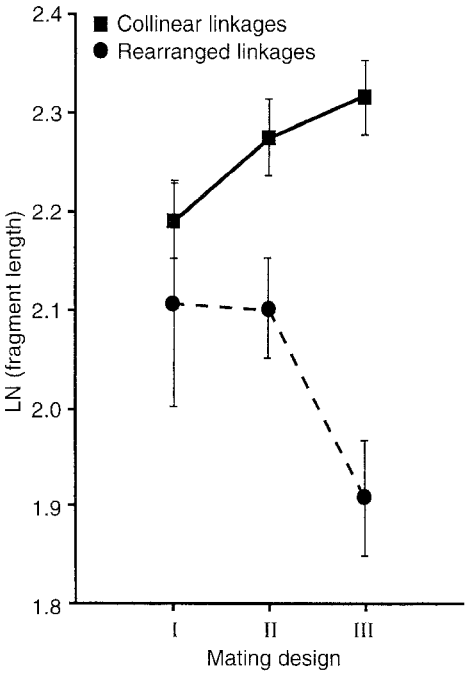


Fig. 3 Interaction plot of transformed fragment lengths for the three mating designs and the collinear and rearranged portions of the genome. Standard error bars are shown

Table 3 Two-way ANOVA of natural-log transformed fragment lengths for the entire, collinear, and rearranged portions of the genome

Source	*df*	SS	MS	*F*	*P*
Overall:					
Mating design	2	1.48	0.74	1.11	0.3305
Genomic region	1	12.72	12.72	19.01	0.0001
M. design × genomic r.	2	5.33	2.67	3.99	0.0187

Fig. 2 Individual graphical genotypes of representative individuals for introgressed regions in each of the seven collinear linkages between *H. annuus* and *H. petiolaris*. Black bars indicate regions flanked by two introgressed *H. petiolaris* markers. Regions harboring recombination sites are indicated by a *gray-scale*, with the intensity of shading based on the genotype of flanking loci. Because of dominance, we were often unable to determine whether the *H. petiolaris* markers or segments were present in the homozygous or heterozygous condition

Table 4 Pairwise comparisons of the effect of mating design on ln-transformed fragment lengths for each linkage group by one-way ANOVA. Significant differences in fragment lengths between designs were tested using the sequential Bonferroni method (experiment-wise error rate α=0.05) and are indicated by an asterisk

Mating design	Mean	SE	*n*	*P*		
				I × II	I × III	II × III
Linkage group A				0.5345	0.7338	0.7417
Design I	2.5915	0.1299	34			
Design II	2.4951	0.0962	62			
Design III	2.5398	0.0858	78			
Linkage group B				0.0059	0.1331	0.1239
Design I	1.1538	0.0879	56			
Design II	1.5321	0.0981	45			
Design III	1.3418	0.0836	62			
Linkage group C				0.2558	0.6282	0.5102
Design I	2.4433	0.0425	103			
Design II	2.5124	0.0423	104			
Design III	2.4729	0.0436	98			
Linkage group D				0.0201	0.9432	0.0088
Design I	2.5940	0.1622	23			
Design II	2.0214	0.1470	28			
Design III	2.6044	0.1187	43			
Linkage group E				0.0022	0.0019	0.5737
Design I	1.1014	0.0487	64			
Design II	1.2768	0.0466	70			
Design III	1.3224	0.0516	57			
Linkage group F				0.0188	0.4640	0.1679
Design I	2.7776	0.0828	86			
Design II	2.5497	0.0676	129			
Design III	2.6962	0.0720	114			
Linkage group HK				[a]	[a]	$\ll$0.0001*
Design I	2.4329	0.1467	15			
Design II	0.1733	0.2842	4			
Linkage group IJ				[a]	[a]	0.0049
Design II	2.3361	0.0920	36			
Design III	1.8477	0.1381	16			
Linkage group L				0.1296	0.0862	0.5553
Design I	2.3731	0.0294	32			
Design II	2.4119	0.0238	49			
Design III	2.4380	0.0294	32			
Linkage group M				[a]	[a]	0.3524
Design II	0.5785	0.2632	4			
Design III	10.467	0.3039	3			
Linkage group NO	(no variance)					
Linkage group PWG				[a]	0.0006*	[a]
Design I	1.0296	0.3050	3			
Design III	2.1462	0.0645	67			
Linkage group RQ	(no variance)					
Linkage group S				0.2580	0.4741	[a]
Design I	2.6954	0.0591	55			
Design II	2.8291	0.0524	70			
Design III	2.7683	0.0561	61			
Linkage group V				NA	0.5059	0.4540
Design I	1.9021	0.0154	22			
Design II	1.9021	0.0147	24			
Design III	1.9170	0.0111	42			

[a] Comparison not made because there was no introgression into the linkage group for one of the mating designs

creases (Fig. 4). Overall, introgressed fragments were much smaller on average (mating design I, $\bar{x}$=11.6±0.42; mating design II, $\bar{x}$=12.4±0.35; and mating design III, $\bar{x}$=12.4±0.34) than those predicted by theoretical models (26–33 cM; Hanson 1959a, 1959b).

Total length of introgressed regions

Differences in the total length of introgressed fragments per individual (Table 5) were highly significant for comparisons of mating design I ($\bar{x}$=95.8 cM) with mating de-

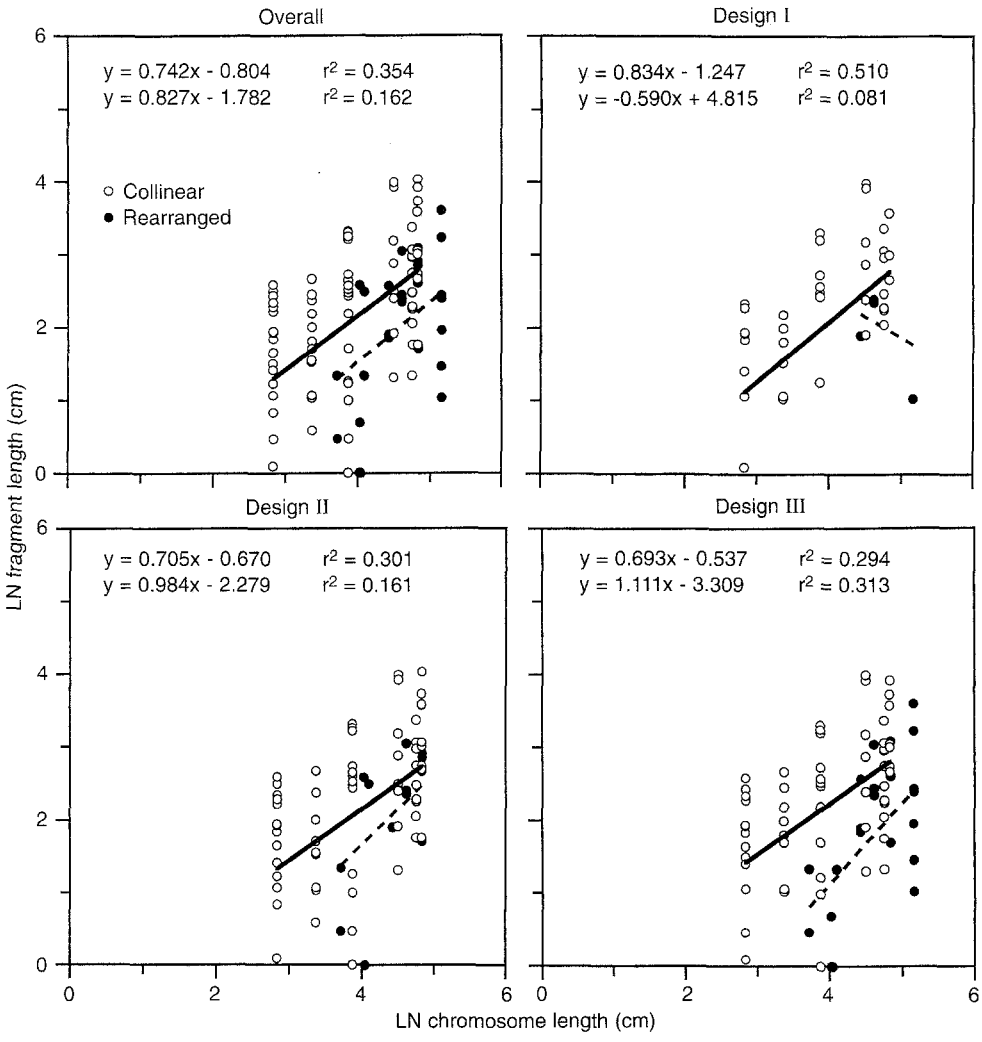


Fig. 4 Linear regressions of the relationship between ln(chromosome map length) and ln(introgressed fragment length) in the collinear and rearranged portions of the genome. Analyses are presented for each mating design and the pooled data from all three. Formulas and r^2 values for the regressions are given in each graph with the formula for the collinear portion always appearing above that for the rearranged portion. *Solid regression lines* are for the collinear linkage groups and *dashed lines* are for the rearranged linkages. Regression lines have not been extrapolated beyond the data

Table 5 Pairwise comparisons of the effect of mating design on the total length of introgressed fragments per individual using one-way ANOVA. Significant differences were tested using the sequential Bonferroni method (experiment-wise error rate α=0.05) and are indicated by an asterisk

Item	Mean (cM)	SE	*n*	*P*		
				I × II	I × III	II × III
Overall				<0.0001*	<0.0001*	0.2920
Design I	95.8	3.99	58			
Design II	148.8	4.06	56			
Design III	158.3	4.06	56			
Collinear				<0.0001*	<0.0001*	0.1274
Design I	87.2	3.79	58			
Design II	119.9	3.77	56			
Design III	127.9	3.50	56			
Overall				<0.0001*	<0.0001*	0.4709
Design I	8.6	1.11	58			
Design II	28.9	1.41	56			
Design III	30.4	1.82	56			

signs II ($\bar{x}$=148.8 cM) and III ($\bar{x}$=158.3 cM). Similar results were observed when the comparisons were restricted to either the collinear or rearranged portion of the genome: mating design I differed significantly from the other two mating designs, but no differences were observed between mating designs II and III (Table 5). Thus, mating design does significantly affect the total length of introgressed fragments for the entire, collinear, and rearranged portions of the genome.

Discussion

Chromosomal sterility factors

As was previously reported for mating design I (Rieseberg et al. 1995b), the primary factors influencing patterns of introgression between *H. annuus* and *H. petiolaris* are chromosomal rearrangements. A smaller proportion of

markers introgress into the rearranged linkages, and those fragments that do introgress are smaller in the rearranged linkages (Tables 1, 3; Figs. 1, 4). These observations are not surprising given that the two species differ by a minimum of seven interchromosomal translocations and three inversions (Rieseberg et al. 1995a), and these rearrangements have been shown previously to be strongly correlated with the pollen fertility of first-generation sunflower hybrids (Chandler et al. 1986). Moreover, Quillet et al. (1995) have recently mapped pollen viability in interspecific hybrids between *H. annuus* and *H. argophyllus* to three genomic regions that contain translocation break-points. Thus, the results reported here are concordant with empirical and theoretical evidence that suggests that chromosomal structural rearrangements reduce effective recombination rates within rearranged linkages by selection against recombinant gametes (Hanson 1959a, b; Grant 1981; Tadmor et al., 1987). This leads to lower rates of introgression within these regions of the genome.

Genic sterility 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 also affect rates of introgression in *Helianthus*. In fact, 38% of the markers from the collinear portion of the genome did not introgress in any of the three mating designs (Fig. 1; Table 1). 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 likely that selection against certain *H. petiolaris* alleles in a *H. annuus* genetic background, combined with linkage, inhibits introgression within the collinear portion of the genome. Moreover, because introgression is inhibited in the same linkage blocks in all three mating designs (Fig. 1), it is possible to estimate the number of genetic intervals that contribute to lowered hybrid viability or fertility. A minimum of 14 genetic intervals appear to contribute to hybrid unfitness within the collinear portion of the genome alone. The number and 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 presence of genomic intervals where introgression is reduced or absent is often reported in map-based studies of introgression (e.g., Jena et al. 1992; Williams et al. 1993; McGrath et al. 1994; Garcia et al. 1995; Wang et al. 1995). Presumably, many of these genomic regions harbor genes that contribute to reproductive isolation. Likewise, strong segregation distortion is often observed in interspecific crosses, suggesting that many genes are negatively selected in hybrids. For example, Zamir and Tadmor (1986) report segregation distortion in 54% of loci from interspecific crosses of lentil, pepper and tomato, compared to only 13% in intraspecific crosses.

Perhaps the most surprising result reported here, however, was the significantly higher than expected rates of introgression observed for 5–6% of *H. petiolaris* markers (e.g., *C-122, C-181, F-476, F-220*, and *S-226*; Fig. 1). These markers appear to be linked to genes that interact favorably in a new genetic background (*H. annuus)*, a hypothesis which is discussed in detail in Rieseberg et al. (1996). A similar hypothesis has been suggested to account for the common persistence of several regions of *Gossypium hirsutum* chromatin in a *G. barbadense* genetic background (Wang et al. 1995). Alternatively, the high frequency of these *H. petiolaris* markers may be due to gene conversion or meiotic drive (Arnold et al. 1988; Rieseberg et al. 1995a). However, these explanations seem unlikely since the high-frequency markers represent 10 of the 17 linkage groups and show significant linkage to adjacent markers.

Introgressed fragment sizes

The lack of significant differences among the three mating designs in overall mean introgressed fragment lengths (Table 2) is not surprising given that all three designs incorporate the same number of sib-cross and backcross generations. The significantly reduced size of introgressed fragments from rearranged linkages is suggestive, however, of strong selection against larger fragments in this portion of the genome. Many meiotic products resulting from crossing-over with rearranged linkages, particularly those generating large fragments, will carry deficiencies and duplications and thus be inviable. Double crossovers involving short fragments will be least likely to create meiotic problems and this expectation is in accordance with the shorter fragment length found in the rearranged linkages.

Significant differences in introgressed fragment lengths for different designs were observed only for two linkage groups (HK and PWG), both from the rearranged portion of the genome. In both cases, only a small number of markers introgressed, and the apparent differences in fragment lengths appear to be an artifact of uneven marker distribution on the linkage maps, rather than an effect of mating design.

Hanson (1959a, b) provided theoretical distributions of the lengths of parental linkage blocks after n generations of backcrossing or sib-mating. For mating designs involving two generations of sib-mating and two generations of backcrossing, such as those analyzed here, theory predicts fragment sizes averaging 26–33 cM for chromosomes ranging from 50 to 200 cM in length. These predicted lengths are much larger than the average fragment sizes reported here (12.2 cM). One explanation for this is that we used the map distances of *H. annuus*, the recipient parent, rather than *H. petiolaris*, the donor parent, to estimate fragment lengths. Because recombination rates in *H. petiolaris* are about 1.6-times higher that those of *H. annuus* (Rieseberg et al. 1995b), this could have a significant impact on estimated fragment lengths. However, even when the fragment sizes are adjusted upwards for *H.*

petiolaris recombination rates (12.2×1.6=19.5 cM) they still fall below expectations. Possibly, longer fragments are more likely to contain negatively selected loci and would be selected against in hybrids and backcrosses. Alternatively, recombination rates may be higher in hybrids than in the intraspecific mapping populations. This latter explanation seems unlikely, however, since recombination rates are typically reduced rather than enhanced in crosses between genetically divergent taxa such as maize/teosinte (Doebley and Stec 1993), tomato species (Paterson et al. 1988), rice species (Causse et al. 1994), and sunflower species (Quillet et al. 1995). Lower than expected fragment sizes (Jena et al. 1992) have also been reported for introgressed segments from a wild rice species (*Oryza officinalis*) into domesticated rice (*O. sativa*) and were attributed to a "non-conventional recombination mechanism."

Although introgressed fragment lengths were shorter than predicted by theory, the expected increase in fragment size with increasing chromosome map length was observed (Fig. 4). Thus, interactions between selection, recombination rate, and chromosome map length appear to control introgressed fragment sizes in crosses between *H. annuus* and *H. petiolaris*.

Breeding implications

Population genetic theory suggests that mating designs employing one or more generations of sib-crossing or selfing prior to backcrossing are more effective than backcrossing alone for moving alleles across linkage groups where effective recombination rates are low, such as chromosomally divergent linkages (Hanson 1959a, b; Wall 1970). Thus, mating designs II and III , where sib-crossing precedes backcrossing, should be more effective at moving alleles across the species barrier than mating design I, in which the backcross generations precede sib-mating. Furthermore, these differences should be particularly noticeable in the rearranged part of the genome where recombination rates are low.

As predicted, mating designs II and III were significantly more effective at moving alleles across the species barrier than mating design I (Tables 2, 5). Disparities in the total number of introgressed markers were largely due to differences in the rearranged portion of the genome (Table 2). However, estimates of overall introgression based on the total length of introgressed fragments per individual revealed that mating designs II and III significantly enhance overall introgression in both the collinear and rearranged portions of the genome. Thus, breeding strategies that include sib-crossing or selfing, in combination with backcrossing, do significantly increase the effectiveness of gene transfer across complex genic or chromosomal sterility barriers. A similar result has previously been reported for corn (Horner 1968) and *Phaseolus* (Wall 1970), where sib mating or selfing, in conjunction with backcrossing, greatly enhanced heterospecific genome recombination, a prerequisite for introgression.

Nonetheless, even in mating design III, where two generations of sib-mating were conducted prior to backcrossing, the proportion of the *H. petiolaris* genome introgressed into *H. annuus* was still quite low, particularly in the rearranged portion of the genome (Fig. 1; Table 1). Undoubtedly, additional generations of sib-mating would enhance overall rates of introgression since this would allow additional donor alleles to recombine into the recipient genome before they are eliminated by selection against alleles with which they were initially associated. However, it might be more efficient to employ marker-assisted introgression (Tanksley et al. 1989) to enhance the introgression of specific traits or to ensure genome-wide introgression. Either strategy would be more effective than recurrent backcrossing, which appears to be woefully inadequate for moving alleles across complex reproductive barriers.

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