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Long-term introgression of crop genes into wild sunflower populations

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Abstract Hybrids between cultivated and wild sunflowers (*Helianthus annuus*) are frequently reported. As much as 42% of progeny from wild plants near cultivar fields are hybrids, and cultivar genes have been shown to persist in wild populations at least five generations. We report the effects of up to 40 years of persistent cultivar gene flow on the genetic structure of three wild *H. annuus* populations that are adjacent to cultivated fields. Eighteen cultivar-specific markers were surveyed in a total of 115 individuals. We also developed cultivar-based genetic maps for the markers and used them to see if marker patterns in the wild populations were consistent with introgression and if selection was acting jointly on unlinked loci. High levels of crop specific markers were detected in all three wild populations, ranging from 0.315 to 0.382, on average. All 115 plants had at least 1 cultivar marker. Introgression is the most likely explanation for the presence of crop-specific markers in the sympatric populations because (1) the markers were either not present or found at much lower levels in four allopatric populations, (2) markers that were genetically linked in the cultivar invariably showed linkage disequilibrium patterns in the sympatric populations consistent with introgression, and (3) homology tests ruled out convergent evolution as an alternate explanation. Hence, introgression of cultivar loci was widespread, and the sympatric wild populations have been replaced by advanced generation hybrids. Unlinked, introgressed markers did not show significant linkage disequilibria, indicating a lack of epistatic associations among introgressed markers. Hence, transgenes in cultivated sunflowers should readily introgress into sympatric wild populations, and their fate will be determined primarily by their fitness effects on the wild plants.

Key words *Helianthus annuus* · Hybridization · Introgression · Random amplified polymorphic DNA · Transgene escape

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Introduction

Hybridization between crops and their wild/weedy relatives has been suggested as the primary risk of commercializing genetically engineered crops (Colwell et al. 1985; Ellstrand 1992; Goodman and Newell 1985; Linder and Schmitt 1994; Tiedje et al. 1989). Many crop species are grown where sexually compatible relatives occur (Ellstrand and Hoffman 1990), and many transgenic traits (e.g., disease, insect, or herbicide resistance) may confer significant fitness advantages to weeds in some environments. Although crop-wild hybridization has been reported frequently (e.g., Brubaker and Wendel 1994; Kirkpatrick and Wilson 1988; Doebley 1984; Robert et al. 1991; Jörgenson and Anderson 1994; Klinger et al. 1991; Langevin et al. 1990; Arriola and Ellstrand 1996; Santoni and Bervillé 1992; Arias and Rieseberg 1994), most studies have documented only first-generation hybrids (although see Doebley et al. 1984; Brubaker and Wendel 1994; Whitton et al. 1997). Because introgression of crop genes beyond F_1 hybrids might be prevented or slowed by hybrid sterility

or breakdown in early hybrid generations (Barton and Hewitt 1985), the demonstration of introgression of crop genes into wild populations is important. A genetic barrier to introgression could lower the risk of transgene escape from crops to wild relatives, whereas the lack of such a barrier could lead to substantial introgression and a high risk of transgene persistence in wild populations.

To detect hybridization, many of the earlier investigators of crop-wild hybridization used phenotypic characters that were likely to be under significant selective pressure. This made it difficult to determine whether characters found in crops and wild relatives were caused by introgressive hybridization or by convergent evolution, phenotypic plasticity, or common descent (National Research Council 1989; Small 1984). These problems can be largely eliminated if molecular markers are used (Rieseberg and Wendel 1993). Most molecular markers do not exhibit plasticity and are unlikely to be similar due to convergent evolution. Joint retention of markers by common descent can be distinguished from introgression if marker profiles for wild populations that have and have not had opportunities to hybridize with a crop are compared.

Genetically mapped markers are even more powerful for distinguishing introgression from common descent. In the absence of selection, one would expect significant linkage disequilibria between linked markers that were present due to recent introgression but not if they were due to common descent (Doebley and Wendel 1989; Rieseberg and Wendel 1993). Mapped markers also allow one to determine how patterns of gene flow are affected by selection (Rieseberg et al. 1996). In particular, one can test whether unlinked markers show significant associations (linkage disequilibria) as a measure of potential selection on coadapted sets of genes.

Cultivated and wild sunflowers (*Helianthus annuus* L.) are an excellent system in which to study the long-term effects of hybridization between crops and their wild relatives. Domesticated and wild/weedy *H. annuus* grow side-by-side in many locations and overlap in flowering time. Both crop and wild flowers are pollinated predominantly by honey bees and larger bees (Heiser 1976) and bear large achenes with no adaptations for long-distance dispersal. Artificial hybridization experiments have shown that the domesticated sunflower produces fertile hybrids when crossed with wild *H. annuus*. Because wild sunflowers are self-incompatible and the domesticated sunflower is self-compatible, it is likely that gene flow from the crop to wild populations is favored. Earlier work showed that up to 42% of progeny from wild plants near cultivar fields were first generation hybrids (Arias and Rieseberg 1994; Whitton et al. 1997). Nonetheless, wild sunflower populations are sufficiently widespread that populations can be found which have had little or no opportunity for hybridization with cultivated relatives, allowing one to distinguish introgression from common descent.

Whitton et al. (1997) showed that cultivar genes can persist in wild populations up to five generations following the initial hybridization event. Here, we extend their study by describing the effects of up to 40 years of persistent cultivar gene flow on the genetic structure of three wild *H. annuus* populations that grew adjacent to cultivated fields. We employed genetically mapped random amplified polymorphic DNA (RAPD) markers to provide assurance that we had detected introgression and to document any effects of selection on marker introgression.

Materials and methods

Plant materials

Three wild populations of *H. annuus* that had been in contact with cultivated sunflowers between 20 and 40 years were selected for study: Estavan, Saskatchewan, Canada (*Seiler* 2318); Lake Metigoshe, Manitoba, Canada (*Seiler* 2328); and Fargo, North Dakota, USA (*Seiler s. n.*). Sunflower production records indicate that the Estavan and Lake Metigoshe populations have been in contact with cultivated sunflowers since the late 1950s or early 1960s (30–40 years), whereas the Fargo population has been in contact with cultivated sunflowers for approximately 20 years. Actual exposure to pollen carrying some of the crop markers we assayed may have been less than 15 years since we chose markers in commercial hybrid 'USDA 894', which has only been used since the early 1980s. Nonetheless, due to the close genealogical relationship among oilseed lines cultivated in the USA, over the past 40 years, many of the markers assayed are diagnostic for all public oilseed lines (Arias and Rieseberg, unpublished data).

For each of the three sympatric wild populations, single, mature heads were collected from 30–100 individual plants at irregular intervals along a transect. Individuals that appeared to be first-generation crop-wild hybrids were excluded from our analysis because we wanted to measure rates of introgression rather than production of F_1s. Achenes from the remaining heads were pooled and randomly chosen for analysis.

To ensure that the markers employed were cultivar-specific, we analyzed 30 DNA samples from each of four wild *H. annuus* populations (120 individuals total) that were allopatric with the cultivated sunflower: A3 and A21 (locality data in Rieseberg et al. 1988), AT6 (locality data in Rieseberg et al. 1990), and *Rieseberg* 1238. A3, A21, and AT6 were collected from California, Missouri, and Texas, respectively, whereas *Rieseberg* 1238 is from the Cedar Point Biological Research Station in western Nebraska. The populations' broad geographic distribution increased the probability that markers found solely in the cultivar were either unique to the cultivar or found in wild populations at very low levels.

To map cultivar-specific markers, a single individual of 'USDA 894' was selfed, and 116 F_2 progeny were used for mapping. This crossing design allowed us to map markers that are heterozygous in 'USDA 894'.

DNA isolations

For the sympatric wild populations, DNA was isolated from whole seedlings using a CTAB microprep (Rieseberg et al. 1993). DNA samples from the cultivar and the mapping populations were isolated from 1 g of fresh leaf tissue using a CTAB miniprep (Rieseberg

et al. 1993). DNA samples from leaf tissue were further purified using the ELU-QUIK™ DNA Purification Kit (Schleicher and Schuell) and then quantified on a fluorometer. Samples were diluted to 10 ng DNA/μl. DNA samples from the allopatric populations were obtained from prior studies of sunflower evolutionary genetics (Rieseberg et al. 1990; 1993).

RAPD marker surveys

Purified DNA samples from the two inbred lines that combine to form 'USDA 894' (CMS 89 and RHA 274), individual DNA samples from two allopatric populations, and a bulked sample of ten allopatric individuals were surveyed for 331 RAPD primers (Williams et al. 1990). The primers were obtained from the University of British Columbia Biotechnology Laboratory (primers 101–300, 329, 331, 337, 343, 345, 348, 376, 431, 437, 450, 474) and Operon Technologies (primer kits A–F). Markers specific to either CMS 89 or RHA 274 or found in both but not the wild plants were then tested in 30 individuals from each of the four allopatric populations of wild *H. annuus*. Eighteen markers were either completely absent in the four allopatric populations or were present at frequencies of less than 1% (1/120). Of these, 8 markers were present in both CMS 89 and RHA 274, so they could not be mapped. The 10 remaining markers were specific to either CMS 89 or RHA 274 and thus mappable. We also identified 70 mappable markers that were not cultivar-specific. All 80 mappable markers were screened in the F_2 mapping population ($n = 116$), allowing estimation of the genomic location of the 10 cultivar-specific markers. Finally, we surveyed between 37 and 40 individuals from each of the sympatric wild populations ($n = 40$, *Seiler s. n.*; $n = 38$, *Seiler* 2318; $n = 37$, *Seiler* 2328) for all 18 cultivar-specific markers.

RAPD amplifications followed the general procedure of Williams et al. (1990). Amplifications were performed 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 $MgCl_2$, 30 mM Tricine, 50 mM KCl, 100 μM each dNTP, 5% acetamide, 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.

Homology tests

We used restriction fragment analysis to test whether co-migrating RAPD fragments from the cultivar and each of the three sympatric wild populations were homologous. We removed a plug of each fragment from gels using a Pasteur pipet. Each cylindrical plug was placed in 300 μl 0.1 × TE and heated at 55°C for 3 h. Ten-microliter aliquots of the eluted fragment were re-amplified following the amplification protocol described above, except that the amplifications were conducted in a total volume of 50 μl. The re-amplified fragments were then digested with two restriction endonucleases that have four base-pair recognition sequences, *Hae*III (GC/CC) and *Hin*fI (G/ANTC). Fragments with identical restriction profiles for both enzymes were considered homologous. All 18 cultivar-specific RAPD fragments were tested for homology in this manner.

Data analysis

Partial linkage maps were generated for the 116 progeny by 80-marker data matrix using MAPMAKER version 3.0 (Lander et al. 1987). A threshold LOD score of 5 and a recombination limit of 0.28 were used to detect linkage, whereas a LOD score of 2 was employed to determine gene orders. Because of dominance and use of an F_2 mapping population, two complementary maps were generated. One describes the linkage relationships among markers found in the inbred line CMS 89, whereas the other describes linkage relationships among markers from RHA 274.

We tested whether the rates of introgression in the three sympatric populations differed by performing one-way analysis of variance on the proportion of individuals into which each marker introgressed in each population (ANOVA, Wilkinson 1990). Proportions were arc-sine square-root transformed before analysis (Sokal and Rohlf 1981). Residuals were checked for violations of normality and heteroscedasticity.

In the sympatric populations, we tested for linkage disequilibrium between pairs of markers by running bootstrap simulations of association for all possible pairwise combinations ($n = 153$) of the 18 cultivar-specific markers. Simulations were run for each of the three populations and for the pooled marker data. Pooling across the three populations gave us more power to detect weak, but significant, associations. The procedure for the simulations was identical to that described in Rieseberg et al. (1996). Using the observed number of introgressions for each marker, we randomly associated each pair of markers 10,000 times. For each of the 10,000 simulations of each marker pair, the simulated degree of association (ρ_E) was compared with the observed degree of association (ρ_O). The proportion of simulated associations having ρ_E values equal to or greater than ρ_O was the probability that the observed association occurred by chance. Since 153 two-way comparisons were made for each population and the pooled data, we controlled the experiment-wise error rate for each set of tests by rejecting the null hypothesis only if the P value was $\leqslant 0.0065$. This α-level ensured that the associations determined to be significant had less than 1 chance in 153 of occurring by chance.

Results

Crop-specific RAPD markers

Of the 18 markers chosen for analysis of crop-wild introgression 14 were absent in all 120 individuals of the wild allopatric populations of *H. annuus*. The remaining 4 markers (*267-0.7*, *150-0.75*, *B20-1.05*, and *223-1.2*) were found in single individuals ($1/120 = 0.0083$). These 4 markers occur at such low frequencies in allopatric populations that they can reliably contribute to estimates of crop-wild introgression. In the absence of introgression, we would expect each of these 4 markers to occur less than once in the 115 individuals we surveyed from sympatric populations. The markers' cumulative probability of appearing is only 0.033, indicating that without introgression, they should appear less than four times (3.8) overall.

Introgression

The average frequency of the 18 cultivar-specific markers was high in all three sympatric wild populations, ranging from 0.315 ± 0.137 per maker ($\bar{x} \pm$ SD) in the Estavan population to 0.382 ± 0.170 per marker in the Lake Metigoshe population. Analysis of variance did not detect differences in the rates of introgression in the three populations ($P = 0.145$, $F_{2,112} = 1.96$), so we

could not reject the null hypothesis that wild populations have equal amounts of introgression when in contact with cultivated plants for different periods of time. All markers introgressed at least once in all three populations (Fig. 1). The 4 markers found once in the allopatric populations were present in the sympatric populations at levels much higher than expected (Overall proportions: *150-0.75* = 0.495; *223-1.2* = 0.390; *267-0.7* = 0.377; *B20-1.05* = 0.407) (Fig. 1), strongly suggesting that their presence in the three sympatric populations was principally due to introgression.

Introgression patterns of individual markers differed (Fig. 1); however, only marker *182-0.52*, which occurred at very low levels, consistently introgressed at rates significantly different from the means of all three populations ($P < 0.05$ in all populations). *182-0.52* may be physically linked to a negatively selected cultivar trait such as loss of seed dormancy or monocephaly. Alternatively, it may have been in lower frequency in the adjacent cultivar populations.

All 115 plants surveyed had at least one cultivar marker (Fig. 2), although the number of markers introgressing per individual varied substantially. The proportion of cultivar markers found in individuals in the three populations was high, averaging between 0.315 ± 0.114 in the Estavan population and 0.381 ± 0.141 in the Lake Metigoshe population. One individual carried 14 of the 18 cultivar markers.

Homology tests

Restriction digestions confirmed homology within each of the 18 sets of co-migrating, cultivar-specific fragments. This result suggests that the fragments scored were derived via cultivar-wild introgression rather than by convergent generation of nonhomologous DNA fragments of similar length.

Linkage mapping

Analysis of the 80-marker by 116-progeny data set generated two complementary maps. The first map, which represents linkage relationships among 41 markers exclusive to CMS 89, comprises six linkage groups of 2 or more markers (Fig. 3). Fifteen markers were unlinked. The CMS 89 map covers approximately 479 cM or about one-third of the *H. annuus* genome (Rieseberg et al. 1995; Berry et al. 1995; Gentzbittel et al. 1995). Cultivar-specific markers *160-3.3* and *A18-1.0* map to linkage group *CMS5* and are separated by only 11.5 cM (Fig. 3). Cultivar-specific markers *E3-0.6*, *223-1.2*, and *267-0.7* map to linkage group *CMS2*. However, these markers are more loosely linked, being separated by map distances of 40.4 cM or greater. Cultivar-specific markers *E12-1.7* and *163-2.1* are unlinked to the markers comprising the map.

The second map, which represents linkage relationships among the 39 markers exclusive to RHA 274, is comprised of eight linkage groups and 6 unlinked markers. It covers approximately 489 cM, 10 cM more than the CMS 89 map. Cultivar-specific markers *167-0.8*, *182-0.52*, and *266-1.0* map to linkage groups *RHA8*, *RHA6*, and *RHA1*, respectively.

Linkage disequilibria

Only four pairs of crop-specific markers exhibited significant associations in all three sympatric populations and in the combined analysis of all populations:

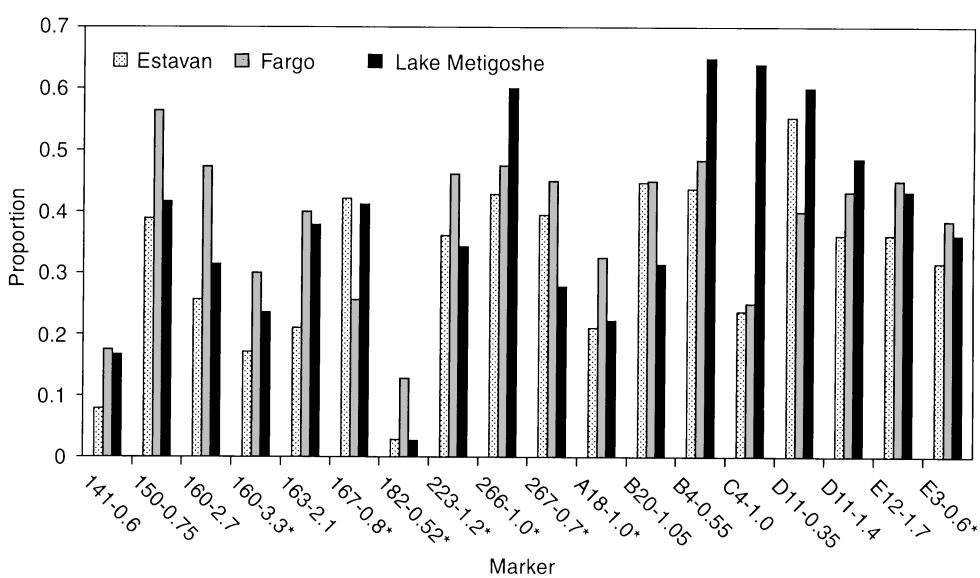


Fig. 1 Proportions of individuals carrying each of 18 crop-specific markers. Results are given for three sympatric populations of wild *H. annuus*. RAPD marker names include the primer designation and the size of the segregating fragment in kilobases. Marker designations followed by an *asterisk* were mapped to linkage groups (see text and Fig. 3)

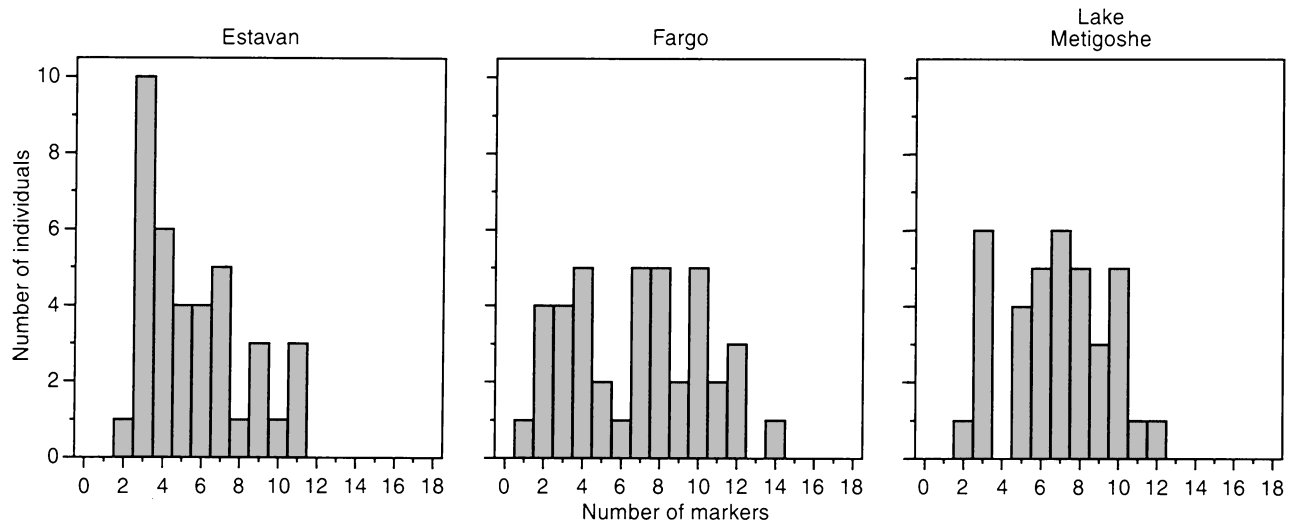


Fig. 2 Histograms of the numbers of individuals in which particular numbers of cultivar specific markers were detected. Results are presented for each of the three sympatric populations of wild *H. annuus*. Note that at least 1 marker introgressed into all individuals sampled

160-3.3/A18-1.0 ($P < 0.0001$ in all populations), *E3-0.6/223-1.2* ($P \leqslant 0.0005$ in all populations), *E3-0.6/267-0.7* ($P \leqslant 0.0038$ in all populations), and *223-1.2/267-0.7* ($P < 0.0001$ in all populations). Each pair is genetically linked in the cultivar (Fig. 3), suggesting that physical linkage rather than selection or breeding history is responsible for the associations observed. For example, in the three sympatric populations, 85-100% of the individuals possessing marker *160-3.3* or *A18-1.0* had both markers (Fig. 4a). In total, only 2 individuals possessed only 1 of these markers. Because these markers are separated by only 11.5 cM on linkage group *CMS5*, this chromosomal segment was almost certainly transmitted intact from the cultivar to the wild plants.

For the 3 markers on *CMS2*, the marker combinations in the sympatric populations occur in precisely the rank order expected for introgression (Fig. 4b). Over half of the individuals carrying at least 1 of the markers possessed all 3 markers. Marker pair *223-1.2/267-0.7* (40.4 cM) occurred most frequently, followed by the more distant marker pair, *E3-0.6/223-1.2* (71.3 cM). As would be predicted, no individuals carried markers *E3-0.6/267-0.7* without the intervening marker, *223-1.2*. Finally, as expected, marker *E3-0.6* occurred alone most often, followed by *267-0.7* and *223-1.2*. These results support introgression as the explanation for the presence of these markers in wild populations.

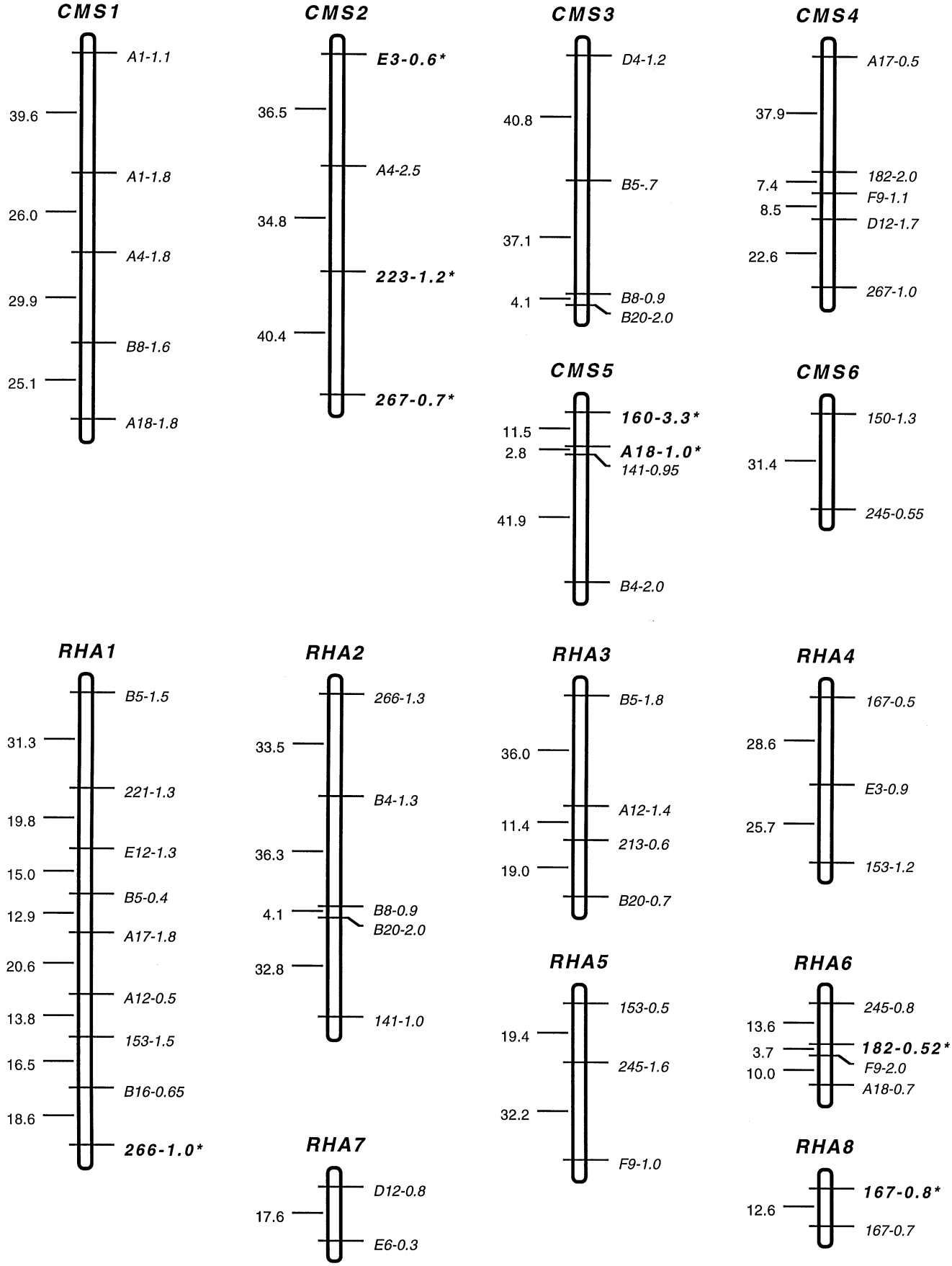
There was no consistent evidence of significant associations between unlinked pairs of markers. The Lake Metigoshe population had no significant associations between unlinked markers. A small number of significant unlinked associations were detected in the Fargo (6) and Estavan (3) populations, but each population had a unique set of marker pairs that were significant. It is possible that the Fargo and Estavan populations had a high proportion of early generation hybrids and that the associations detected were simply the result of chance co-segregation of particular crop chromosomes. Alternatively, there could be unique selective pressures in these populations that maintain certain unlinked associations between chromosomal fragments derived from the cultigen.

Discussion

Crop-wild introgression: empirical evidence

This paper provides unambiguous documentation of extensive long-term introgression of crop genes into sympatric wild populations of sunflower. Most circumstances favor gene flow from cultivated sunflowers to wild populations (Heiser 1976; Arias and Rieseberg 1994; Whitton et al. 1997; Snow et al. 1997), so we anticipated some introgression. However, its extent was unexpected, as was its pervasive effect on the genetic structure of sympatric wild populations. Not only was the average overall frequency of cultivar markers greater than 35%, but every individual in the three populations tested contained at least some cultivar genetic material.

We can think of no plausible alternative to introgression for these results. Use of molecular markers and verification of homology among co-migrating fragments rule out the possibility of convergent evolution. Joint retention of ancestral characters also seems an unlikely explanation due to (1) the nearly complete lack of the markers in four allopatric populations, (2) the extensive multilocus patterns of introgression observed,


CMS1
A1-1.1
39.6
A1-1.8
26.0
A4-1.8
29.9
B8-1.6
25.1
A18-1.8
CMS2
E3-0.6*
36.5
A4-2.5
34.8
223-1.2*
40.4
267-0.7*
CMS3
D4-1.2
40.8
B5-.7
37.1
B8-0.9
4.1
B20-2.0
CMS4
A17-0.5
37.9
182-2.0
7.4
F9-1.1
8.5
D12-1.7
22.6
267-1.0
CMS5
160-3.3*
11.5
A18-1.0*
2.8
141-0.95
41.9
B4-2.0
CMS6
150-1.3
31.4
245-0.55
RHA1
B5-1.5
31.3
221-1.3
19.8
E12-1.3
15.0
B5-0.4
12.9
A17-1.8
20.6
A12-0.5
13.8
153-1.5
16.5
B16-0.65
18.6
266-1.0*
RHA2
266-1.3
33.5
B4-1.3
36.3
B8-0.9
4.1
B20-2.0
32.8
141-1.0
RHA3
B5-1.8
36.0
A12-1.4
11.4
213-0.6
19.0
B20-0.7
RHA4
167-0.5
28.6
E3-0.9
25.7
153-1.2
RHA5
153-0.5
19.4
245-1.6
32.2
F9-1.0
RHA6
245-0.8
13.6
182-0.52*
3.7
F9-2.0
10.0
A18-0.7
RHA7
D12-0.8
17.6
E6-0.3
RHA8
167-0.8*
12.6
167-0.7

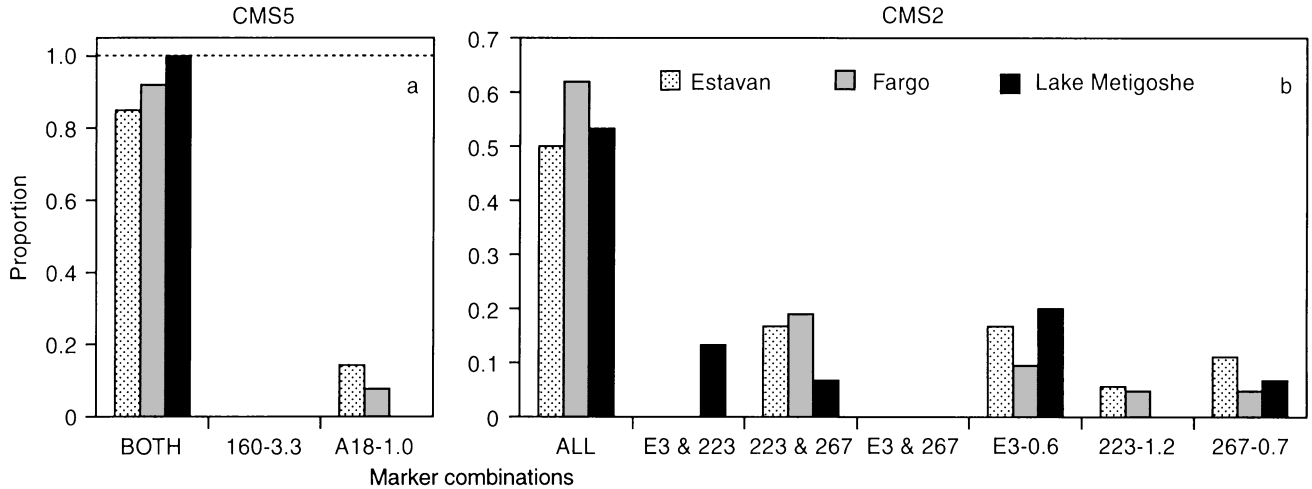


Fig. 4a, b Proportions of individuals containing the possible combinations of crop-specific markers on *CMS2* and *CMS5*. Results are presented for each of three sympatric populations of wild *H. annuus*. For each linkage, only individuals that had at least 1 crop-specific marker were included in the calculations. Note different scales for the two linkage groups. **a** The proportions of individuals into which both *160-3.3* and *A18-1.0* introgressed (*BOTH*) and into which only 1 marker introgressed (*160–3.3* and *A18-1.0*). **b** The proportions of individuals into which all 3 markers (*E3-0.6*, *223-1.2*, and *267-0.7*) introgressed (*ALL*), into which only pairs of markers introgressed (*E3 and 223*, *223 and 267*, *and E3* and *267*), and into which only 1 marker introgressed (*E3-0.6*, *223–1.2,* and *267–0.7*)

and (3) the presence of significant linkage disequilibria among physically linked loci. Because many of the putative introgressants carry multiple, linked cultivar markers from linkage groups *CMS2* and *CMS5*, it is likely that large portions of these chromosomes were transferred intact from cultivated to wild sunflowers. Many cultivated *H. annuus* loci in diverse genomic locations have introgressed and persisted for extended periods of time. For all practical purposes, the sympatric wild populations studied have been replaced by advanced-generation hybrids. Our results support and extend those of Whitton et al. (1997), who showed that 2 cultivar markers remained in a wild *H. annuus* population without significant changes in frequencies for 5 years after a single generation of hybridization.

Introgression of cultivar genes into wild populations has also been clearly demonstrated for corn (Doebley 1984) and cotton (Wendel and Percy 1990; Wendel et al. 1992; Brubaker et al. 1993; Brubaker and Wendel 1994), and putative cultivar-specific allozymes have been found at low frequencies in wild relatives of squash (*Cucurbita pepo*; Decker and Wilson 1987), rice (*Oryza sativa*; Second 1982) and *Quinoa* (*Chenopodium* spp.; Wilson 1990). In *Brassica*, feral populations of *B. campestris* have been shown to contain first-generation backcross individuals that possess a glufosinate tolerance transgene introgressed from oilseed rape, *B. napus* (Mikkelsen et al. 1996).

Lack of evidence for fitness epistasis among introgressed markers

Rieseberg et al. (1996) demonstrated that strong associations were repeatedly maintained between unlinked markers (fitness epistasis) in backcross generations of artificial hybrids between *H. annuus* and *H. petiolaris*. In contrast, we found that only pairs of markers known to be physically linked showed significant associations in all three sympatric populations. Thus, we lack strong evidence for fitness epistasis between any of the markers we examined. Rieseberg et al. (1996) surmised that markers showing strong associations in their study were linked to loci that restore fertility in later generations. Strong selection pressure for restoration of fertility is not present in crop-wild hybrids of *H. annuus* because hybrids frequently show little reduction in fitness relative to the wild parent (Snow et al. 1997). This may account for the lack of fitness epistasis in our study. Alternatively, we may not have examined sufficient crop-specific markers to cover enough of the genome to detect epistatic associations.

Our results indicate the lack of a strong barrier to crop-wild introgression in sunflower. Only 1 of 18 crop-specific markers consistently introgressed at rates lower than the population means. Hence, a transgene will be prevented from introgressing into a sympatric wild population only if it lowers fitness or is tightly linked to

Fig. 3 Genetic linkage maps for *H. annuus* cv 'USDA 894'. Linkage groups derived from the CMS 89 parent are designated *CMS1-6*, whereas those derived from the RHA274 parent are designated *RHA1-8*. Haldane map distances in centiMorgans are listed on the *left* and RAPD markers on the *right* of each linkage group. RAPD marker names include the primer designation and the size of the segregating fragment in kilobases. Cultivar-specific markers are in **bold** print and followed by an *asterisk*

a gene that lowers fitness. Advantageous or neutral transgenes will quickly spread into wild populations, whereas negatively selected transgenes are unlikely to invade wild populations – at least those outside of the immediate area of sympatry.

Snow and Morán-Palma (1997) provided a review of the transgenic traits currently under development in sunflower, which include insect resistance, oil characteristics, and tolerance to diseases. The effects of transgenes for these traits on the dynamics of wild sunflower populations are difficult to predict. It is possible that the invasiveness of wild sunflower populations might increase if a transgenic trait provides ecological release from one or more control factors. However, potential negative consequences should be weighed against the likely benefits of transgenic sunflowers, such as reduced use of pesticides, enhanced yields, and expanded cultivation range.

Crop-wild introgression: theoretical considerations

Unlike sunflowers, some crop-wild hybrid combinations have greatly reduced fertility or viability. However, even in these cases, transgenes are likely to introgress and persist primarily due to their fitness effects in the wild plants. As noted by Harrison (1991), "Genetic isolation must be considered as a property of individual genes (or chromosomal segments), not as a characteristic of entire genomes."A favorable or neutral allele need only recombine into the new genetic background before it is eliminated by selection against the alleles with which it is initially associated (Barton and Hewitt 1985). Thus, selection against hybrids may create a barrier to introgression for negatively selected loci, but the introgression of advantageous or neutral alleles will be slowed down significantly only if they are tightly linked to loci under negative selection. For example, although F_1 hybrids of transgenic, herbicide-tolerant oilseed rape (*Brassica napus*) and wild *B. campestris* had greatly reduced fertility (approx. 35%), fertile BC_1 plants were recovered that carry the transgene (Mikkelsen et al. 1996).

Even linkage with negatively selected loci may not be a major barrier to crop-weed gene flow. Because many crops and their wild relatives have extensive areas of contact, the opportunity for hybridization and, hence, recombination between crop and wild genomes is high. Thus, even tight linkage is unlikely to be a long-term deterrent to transgene escape. Lack of a strong correlation between hybrid fitness and potential for gene dispersal means that proper risk assessment must study both the fitness consequences of individual transgenes in a wild-type background and pollen dispersal distances (Rees et al. 1991). Estimates of early generation crop-wild hybrid fitness are probably of little predictive value concerning transgene escape unless the hybrids are completely sterile.

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