Theor Appl Genet (1997) 95:33–40 © Springer-Verlag 1997

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The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization

Received: 1 November 1996 / Accepted: 17 January 1997

Abstract The development of transgenic plants has heightened concern about the possible escape of genetically engineered material into the wild. Hybridization between crops and their wild relatives provides a mechanism by which this could occur. While hybridization has been documented between several crops and wild or weedy relatives, little is known about the persistence of cultivar genes in wild populations in the generations following hybridization. Wild and weedy sunflowers occur sympatrically with cultivated sunflowers throughout much of the cultivation range, and hybridization is known to occur. We surveyed two cultivar-specific RAPD markers in 2700 progeny in a naturally occurring population of wild *Helianthus annuus* over five generations following a single generation of hybridization with the cultivar. Moderate levels of gene flow were detected in the first generation (42% hybrids at the crop margin) and cultivar allele frequencies did not significantly decline over four subsequent generations. These results indicate that gene flow from cultivated into wild populations of sunflowers can result in the long-term establishment of cultivar alleles in wild populations. Furthermore, we conclude that neutral or favorable transgenes have the potential to escape and persist in wild sunflower populations.

Key words Gene flow · Hybridization · Sunflowers · Transgenes

Communicated by P. M. A. Tigerstedt

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Introduction

Over the last decade, a dramatic increase in the number of requests for field trial permits for transgenic plants has been accompanied by increasing concern about the potential negative effects of the wide-scale commercial release of genetically engineered crops (Kareiva 1993; The Gene Exchange 1993; Seidler and Levin 1994). Potential negative consequences include the possibility that transgenic crops will become feral, or that the transgenes will themselves escape via hybridization with wild or weedy populations of crop relatives (Keeler and Turner 1990; Rees et al. 1991; Raybould and Gray 1994). Escape by either means may have serious agricultural and/or ecological consequences, the severity of which will depend primarily on the nature of the engineered trait and its ability to persist and spread in the environment.

The act of introducing genetically engineered material, in and of itself, is unlikely to result in major changes in life-history traits of crops (Miller and Gunary 1993). Thus, the risk of a transgenic crop escaping cultivation is likely to be highest in crops where non-transgenic varieties have weedy tendencies, or where the engineered trait is likely to improve invasive tendencies (Keeler 1989; Keeler and Turner 1990). This is supported by recent work in which Crawley et al. (1993) found no significant increase in the invasiveness of transgenic oilseed rape (*Brassica napus*) under a wide variety of environmental conditions. It should, however, be noted that the genetically engineered traits carried by these cultivars (resistance to the antibiotic kanamycin and tolerance of the herbicide glufosinate) were not expected to be favorably selected under the conditions of the experiment. This limitation in the design of this and other experiments has been noted by Miller and Gunary (1993) and others (e.g., Kareiva 1993), who state that in order to assess the potential deleterious consequences of escaped transgenic plants, experimental conditions should mimic a plausible scenario

in which the transgenic crop would be selectively favored.

Perhaps a greater area of concern involving the widespread commercial release of transgenic crops is the potential for hybridization with wild or weedy relatives leading to the escape of transgenes into weedy or natural population systems (Goodman and Newell 1985; Ellstrand and Hoffman 1990). A listing of the world's worst weeds reveals that 11 of the 18 most serious weeds are also grown as crops in several countries (Holm et al. 1977; Colwell et al. 1985) and most crops have congeneric weedy relatives (Barrett 1983; Small 1984; Ellstrand 1992). If crops and their weedy relatives co-occur, overlap in flowering time, share pollinators and are cross-compatible, then hybridization has the potential to introduce transgenes into weedy populations (Ellstrand and Hoffman 1990; Keeler and Turner 1990; Darmency 1994; Frello et al. 1995). Although the most highly touted negative consequence of transgene escape via hybridization has been the potential for the increased weediness of wild relatives, concern has also been expressed that the movement of artificially manipulated genetic material into natural population systems may disrupt the evolutionary dynamics of these systems (Ellstrand 1988; Ellstrand and Hoffman 1990; Kareiva et al. 1994). While the perceived risks of transgene escape may differ, whether based on agricultural or ecological concerns, many risk-assessment parameters are common to both perspectives. Among these are the phenotypic effect, mode of inheritance and expression of the engineered character, the likelihood of hybridization between crops and weedy relatives, and of the subsequent persistence and spread of transgenic wild plants (Ellstrand and Hoffman 1990; Jørgensen et al. 1996).

Most commercially released transgenes are likely to be inherited as simple Mendelian single-locus traits (Raybould and Gray 1994), thus facilitating their expression in novel genetic backgrounds (Frello et al. 1995). Certain characters targeted for genetic engineering could potentially contribute to increased fitness, should they become established in weedy populations. Traits such as increased herbicide tolerance, pest or disease resistance, or tolerance to environmental stresses such as drought or salinity, have the potential to increase invasiveness under certain environmental conditions. For example, if tolerance to one or more herbicides is transferred to weedy crop relatives in areas where the herbicides are used to control weeds, then weed control is likely to become more difficult. The impact of the escape of other traits, such as modified seed storage proteins, is less predictable, but seems unlikely to contribute to weed-control problems.

Recently, an increasing number of cases of gene flow from crops into their wild relatives has been documented. Examples include cotton (Brubaker et al. 1993), cucumber (Kirkpatrick and Wilson 1988), maize (Doebley et al. 1984; Doebley 1990), millet (Robert et al. 1991; Till-Bottraud et al. 1992), oilseed rape (Jørgensen and Andersen 1994; Mikkelsen et al. 1996), quinoa (Wilson and Manhart 1993), radish (Klinger et al. 1991, 1992), rice (Oka and Chang 1961; Langevin et al. 1990), sorghum (Arriola and Ellstrand 1996), strawberry (Spira et al. 1996), sugar beets (Santoni and Bervillé 1992; Boudry et al. 1993), sunflower (Arias and Rieseberg 1994), and watermelon (Zamir et al. 1984). In several of these cases, substantial levels of gene flow were observed under conditions commonly encountered in agricultural settings, thus indicating that transgenes have the potential to escape via hybridization with wild or weedy relatives. However, the potential long-term deleterious effects of transgene escape depend on the likelihood of the persistence and spread of transgenes in weedy or natural population systems (Linder and Schmitt 1994; Mikkelsen et al. 1996). Information regarding the likelihood of persistence comes from two types of studies: those that indirectly estimate the likelihood of persistence through studies of hybrid fitness, and those that directly estimate the persistence of cultivar markers in generations following hybridization.

The fitness of first-generation hybrids will provide some indication of the likelihood and rate of spread of transgenes, because extreme hybrid sterility will render transgene establishment unlikely (Linder and Schmitt 1994). Among the few studies that have examined fitness-related traits of crop-weed hybrids (Langevin et al. 1990; Klinger and Ellstrand 1994; Frello et al 1995; Linder and Schmitt 1995; Arriola and Ellstrand 1997; Jørgensen et al. 1996; Mikkelsen et al. 1996; Snow et al. 1996), most have found crop-weed hybrids to be nearly as fit as their wild parent, and only one of these cases reports strong fitness declines among hybrid progeny (Jørgensen et al. 1996). Thus, in most of the cases examined, crop-weed hybrids provide a vehicle for the movement of transgenes into wild populations.

Direct measures of the persistence of cultivar markers in wild species following hybridization are rare (Linder and Schmitt 1994). Luby and McNichol (1995) surveyed wild and feral populations of raspberries (*Rubus idaeus*) in east central Scotland for the presence of traits introduced into cultivars by traditional breeding some 20–30 years ago. They were unable to detect one of the traits, *L1*, which primarily affects fruit size, and detected the other, *s*, encoding spinelessness, at very low frequencies (0.04%). They concluded that while transgene escape from cultivated raspberries may occur, traits with neutral or deleterious effects are unlikely to reach high frequencies in wild relatives. Mikkelsen et al. (1996) provided direct evidence of transgene movement via hybridization from oilseed rape (*Brassica napus*) into its weedy relative, *B. campestris.* They found highly fertile, glufosinate-tolerant plants with the morphology and chromosome complement of *B. campestris* among the first generation backcrossed progeny of populations of F_1 hybrids

grown together with *B. campestris*. Their results suggest that glufosinate tolerance could easily escape into *B. campestris* populations. The use of glufosinate to control weeds in or near oilseed rape fields would provide a strong selective agent for the spread of the transgene in *B. campestris* populations (Mikkelsen et al. 1996). These studies show the potential for the persistence of cultivar genes in later generations of crop-weed hybrids, and indicate the need for additional studies to document levels of persistence of cultivar markers under common agricultural conditions.

The domesticated sunflower (*Helianthus annuus*) is one of only a handful of crops to have originated in North America and appears to have been domesticated from the common annual sunflower (also *H. annuus*) over 4000 years ago (Smith 1989; Crites 1993). Domesticated sunflowers currently represent the second most important source of edible seed oils after soybeans (Fick 1989; Seiler and Jan 1994) and are grown as crops in North America, Argentina, Europe, and in the Commonwealth of Independent States (Heiser 1976; Putt 1978). In North America, the cultivation range of domesticated sunflower overlaps almost entirely with the natural range of wild *H. annuus*. Wild *H. annuus* grows as a weed along roadsides, in pastures, and in agricultural fields throughout much of the Midwestern United States and in California, including fields where the domesticated sunflower is grown. Domesticated and wild *H. annuus* overlap in flowering time, share pollinators, and are cross-compatible in areas of sympatry. These characteristics place sunflowers in a high-risk category in terms of the likelihood of transgene escape (Keeler and Turner 1990). In addition, hybridization between crop sunflowers and their wild relatives is possible and has been documented under experimental settings (Arias and Rieseberg 1994). Using a cultivar-specific isozyme marker, Arias and Rieseberg detected substantial levels of gene flow (27% at the crop margin) among the progeny of wild individuals. Their experiment clearly documented gene flow from crop into wild sunflowers and served as the impetus for this project. The present study was designed to examine the persistence of cultivar genes in a naturally occurring weedy population of *H. annuus* over five generations following hybridization. If a single generation of hybridization can lead to the persistence of cultivar alleles at moderate frequencies in weedy populations, this will suggest that transgenes are also likely to escape via hybridization, persist, and perhaps spread into wild populations of *H. annuus*.

Materials and methods

Field setting and sampling strategy

The population of wild *H. annuus* selected for this study was a naturally occurring stand adjacent to an agricultural field approximately 4 km west of Sacramento, California. The field was planted with cultivated sunflower for the first and only time in 1991. Prior to flowering, we sampled leaves of 60 wild plants and several cultivar individuals (Generation 0) for the presence of cultivar-specific RAPD markers. The frequency of two such markers was assayed in 900 seed progeny in 1991 (Generation 1), 1993 (Generation 3) and 1995 (Generation 5). Achenes were sampled along three parallel transects approximately 100-m apart, perpendicular to the eastern edge of the crop field. Within each transect, seeds heads were collected at three distances from the field edge: 3 m, 200 m and 400 m. At each of these points, single heads from ten individuals spaced approximately 1-m apart were collected. Seven to ten achenes per head were assayed for the presence of each molecular marker.

Laboratory methods

DNA was extracted either from leaf material (Generation 0), seedlings germinated in Petri dishes lined with wet filter paper (Generations 1 and 3), or directly from embryos after the removal of the maternal fruit wall (Generation 5). A scaled-down version of the CTAB method (Saghai-Maroof et al. 1984; Doyle and Doyle 1987), as employed by Rieseberg et al. (1992) with the addition of 1% (W/V) sodium metabisulfite, was used in DNA extractions. DNAs were quantified on a TKO-100 fluorometer (Hoefer Scientific Instruments) and standardized to 10 ng/μl. Surveys of Generation-0-samples for cultivar-specific markers were performed using 30 RAPD primers, and two cultivar-specific RAPD markers were selected: a 1.3-kb fragment amplified with Operon primer A2, and a 0.7-kb fragment amplified with Operon primer B1. These were scored in progeny from each of the three sampled generations. Amplifications were carried out in a 25-μl vol with 5 ng of sample DNA, 10 pmols of primer, in a final concentration of 100 μM of each deoxynucleotide, 30 mM tricine, 50 mM KCl, 2 mM $MgCl_2$, 5% acetamide and 1 units of *Taq* DNA polymerase. Reactions were overlaid with mineral oil and amplifications carried out on MJ Research thermocyclers. Amplifications using the A2 primer were performed in plates on a 96-well model (PTC-100) programmed for an initial denaturation step of 1 min at 94°C, followed by 45 cycles of 40 s at 92°C, 1 min at 48°C and 2 min at 72°C, followed by a final 7-min extension at 72°C. Amplifications using the B2 primer were performed in 0.6-ml microfuge tubes on 60-well thermocyclers programmed for an initial denaturation step of 1 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 10 s at 72°C, followed by a final 7-min extension at 72°C. Amplification products were separated on 1.5% agarose gels in 0.5 TBE buffer, stained with ethidium bromide and visualized under UV light.

Generation-3 DNAs were also assayed for the presence of a cultivar-specific, maternally inherited mitochondrial DNA marker (Rieseberg et al. 1994), to determine if there was evidence of gene flow through seeds from the cultivated field. The mitochondrial DNA-specific marker is characteristic of cytoplasmic male-sterility (CMS) lines in cultivated sunflower, which are used as maternal parents in generating hybrid sunflower lines (male fertility is restored via nuclear restorer alleles in the male parent of these lines). Amplification of cultivar DNAs, combining single primers derived from mitochondrial genes atpA, orfH873R and orfH522, yields two fragments: a 1450-bp fragment and an 870-bp fragment. The larger fragment characterizes an insertion and inversion producing CMS (Köhler et al. 1991). CMS lines have been shown to carry both fragments, while fertile lines carry only the 870-bp fragment. We verified that the cultivated line used in this study carried the CMS marker and then examined the 900 Generation-3 progeny for the presence the 1450-bp cultivar marker. Amplification reactions were performed on MJ Research thermocyclers programmed for an initial denaturation of 1 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 62°C and 2 min at 72°C, with a final extension of 7 min at 72°C. Amplification conditions and electrophoresis were as described above for RAPDs, except that three primers were used in amplification reactions.

Data analysis

For each of the three sampled generations, allele frequencies for each RAPD marker were estimated at nine points in the wild population (three transects × three distances from the edge of the cultivated field). We determined that each of the RAPD markers was heterozygous in the cultivar, because first-generation hybrids carried one, the other, or both markers, and because the two markers occurred in roughly equal frequencies in the first generation. Because a single pollinator carrying a single pollen load is likely to have pollinated many flowers on a single head, the ten achenes sampled from each head represented a non-independent sub-sample of the population. We therefore used the proportion of seed from each maternal plant carrying each marker (observed marker frequency) to estimate allele frequencies in each sub-population. In the first generation following gene flow from the cultivar (Generation 1), allele frequencies were estimated as half of the observed marker frequency in the progeny. This is because all achenes possessing the marker were assumed to be heterozygous. Because of our inability to distinguish between homozygotes and heterozygotes carrying the dominant marker, the following equation was used to estimate allele frequencies in later generations:

$$m = p^2 + 2pq(0.5) + 2pq(0.5p) + q^2p,$$

where m is the expected marker frequency, given a 'marker-present' frequency of p and a 'marker-absent' frequency of q. This assumes that the parental generations giving rise to seeds sampled in Generations 3 and 5 are in Hardy-Weinberg equilibrium at the observed loci, and that allele frequencies in pollen and ovules were equal. This equation arises in the following manner: a frequency of p adults will carry the marker allele. Thus p^2 of the mothers will be homozygous for the marker, $2pq$ will be heterozygous, and q^2 will not have the marker. All of the progeny of mothers that are homozygous for the presence of the marker (p^2) will carry the marker. Among the offspring of heterozygous mothers ($2pq$), half will obtain the marker from their mothers, and a fraction p of those that do not will obtain the marker from the pollen parent. Again, a fraction p of offspring of mothers lacking the marker (q^2) will obtain the marker from the pollen parent. Solving this equation for p, using Mathematica 2.2.1 yields:

$$p = 1 - 0.5\sqrt{4 - 4m}.$$

The invariance property of maximum-likelihood estimators (Mood et al. 1974, p 284) guarantees that the solution of this equation is a maximum-likelihood estimate of the allele frequency.

Data analyses were carried out using Statview 4.5 (1995). The allele frequencies for each sub-population were arcsin square root, transformed to correct for deviations from normality. The transformed allele-frequency data were compared across the two primers over the three distances from the edge of the cultivated field and in the three generations with a three-way ANOVA. We also used a regression analysis to examine changes in allele frequencies over the three sampled generations.

Hybrid frequencies were estimated only in Generation 1 because recombination precludes calculation of hybrid frequencies in later generations. Because both RAPD markers were heterozygous in the cultivars, hybrid frequency in Generation 1 is estimated from each marker separately as twice the observed frequency of progeny carrying the marker.

Results

Evidence of gene flow from the cultivar into the surrounding population of *H. annuus* was observed in all nine sub-populations in all three generations sampled. However, marker frequencies did differ among distances and among transects. For example, in Generation 1, among the three sub-populations collected at 3 m from the edge of the cultivated field, five mothers from one sub-population received some cultivar pollen, as evidenced by the presence of at least one cultivar marker among their progeny. Eight and nine mothers from the other two 3-m sub-populations showed evidence of pollen movement from the cultivar (note that because both cultivar markers were heterozygous, these may be underestimates of the number of mothers receiving cultivar pollen.) These differences most likely reflect differences in pollinator movement along the three transects.

As predicted by pollen-movement patterns (Arias and Rieseberg 1994; Kareiva et al. 1994; Timmons et al. 1996), overall hybrid and allele frequencies were initially highest at the 3-m sampling points closest to the cultivated field (42% hybrids) and dropped off rapidly at 200 m (10% hybrids) and 400 m (4% hybrids; Figs. 1, 2). Allele frequencies tended to even out over the four subsequent generations (Figs. 2, 3). The effect of distance on allele frequency was highly significant ($P<0.0001$; Table 1) and Fisher's paired least-significant-difference tests indicated that differences among all three pairs of distances contributed to this effect.

The results of the ANOVA also indicate a significant effect of generation on allele frequency ($P = 0.0010$; Table 1). Fisher's paired least-significant-difference tests indicated that the effect of generation was mostly attributable to comparisons between Generations 1 and 3 and 2 and 3. Thus, the significance of generations is mostly attributable to the observed increase in allele frequency in Generation 5. A significant interaction between distance and generation ($P = 0.0167$) was obtained from the ANOVA (Table 1). However, the

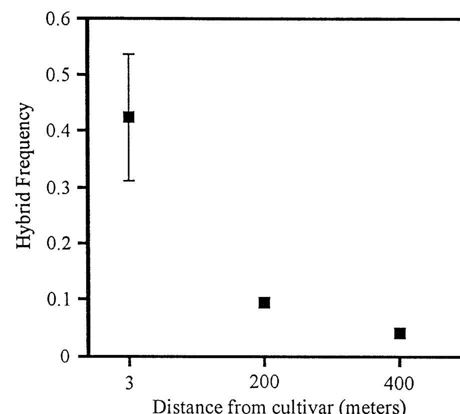

Fig. 1 Hybrid frequencies among the progeny of wild *H. annuus* mothers in the first generation following hybridization at each of three distances from the edge of the cultivated field. Hybrid frequencies were estimated separately for each marker then averaged (see text for details). *Error bars* indicate variances. Where error bars are not shown, they are smaller than the symbols indicating means

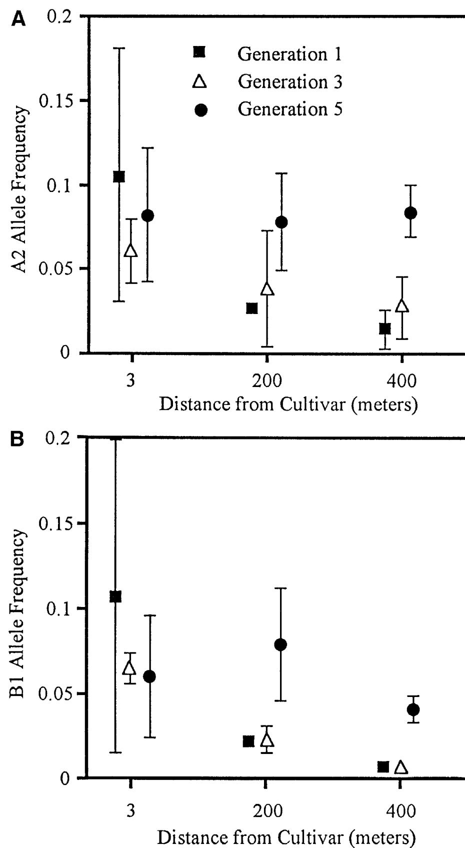

Fig. 2A, B Cultivar allele frequencies in wild *H. annuus* progeny at each of three distances from the edge of the cultivated field in each of the three sampled generations following hybridization. **A** The frequency of the 1.3-kb fragment amplified by Operon primer A2. **B** The frequency of the 0.7-kb fragment amplified by Operon primer B1. *Error bars* indicate variances. Where error bars are not shown, they are smaller than the symbols indicating means

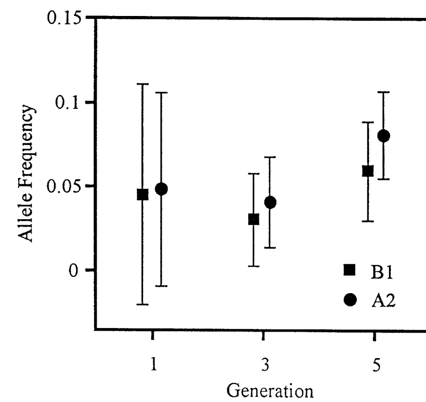

Fig. 3 The frequency of cultivar alleles in wild *H. annuus* progeny averaged over all sampling points for each of the three sampled generations. Regression analysis indicates that allele frequency is not changing over the three sampled generations

Table 1 Results of the three-way ANOVA

	df	*F*-value	*P*-value
Generation	2	8.395	0.0010
Distance	2	14.281	< 0.0001
Marker	1	2.615	0.1146
Generation * distance	4	3.592	0.0145
Generation * marker	2	0.196	0.8232
Distance * marker	2	1.067	0.3545
Generation * distance * marker	4	0.262	0.9002
Residual	36		

regression analysis of generation on overall allele frequency indicated no significant change in marker frequency over the three sampled generations ($r = 0.167$, $P = 0.034$ for the A2 marker; $r = 0.069$, $P = 0.185$ for the B1 marker; $\alpha = 0.025$). This apparent discrepancy between the results of the ANOVA and the regression results from the non-linearity of changes in allele frequency over the sampled generations: allele frequencies decrease from Generations 1 to 3, then increase from Generations 3 to 5 (Fig. 3). The ANOVA detects significant changes among generations, whereas the regression fails to find a consistent trend in allele frequency changes.

The two RAPD markers did not differ significantly in their patterns of introgression into the surrounding field ($P = 0.1146$), indicating that the selective regime on the chromosomal segments containing each markers was not sharply different.

We found no evidence for seed flow from the cultivar into the surrounding wild population. None of the 900 Generation-3 progeny carried the 1450-bp marker characteristic of the cultivar. It is possible, however, that achenes from the cultivar initially invaded the field, but did not contribute to subsequent generations through seeds.

Discussion

An increasing number of studies have documented gene flow from crops into their wild or weedy relatives (Kirkpatrick and Wilson 1988; Langevin et al. 1990; Klinger et al. 1991, 1992; Santoni and Bervillé 1992; Till-bottraud et al. 1992; Boudry et al. 1993; Wilson and Manhart 1993; Arias and Rieseberg 1994; Arriola and Ellstrand 1996; Mikkelsen et al. 1996), thus indicating the potential for transgene escape via hybridization. However, the impact of transgene escape depends on the likelihood of persistence and spread of transgenes in weedy crop relatives. Our results clearly demonstrate that cultivar alleles can escape and persist at moderate frequencies in naturally occurring wild sunflower populations.

We obtained even higher rates of gene flow than were found under experimental conditions by Arias and

Rieseberg (1994). This difference may be attributable to a variety of sources, including the larger size of the cultivated field which would provide a much larger source of pollen compared with the experimental plots. Patterns of gene flow were however similar between the two studies: cultivar allele frequencies were initially highest at the crop margin, and dropped off rapidly as distance from the cultivar increased (compare Generation 1 in our Fig. 2 with Fig. 2 in Arias and Rieseberg 1994). These patterns are expected from the patterns of pollen movement (Arias and Rieseberg 1994; Kareiva et al. 1994; Timmons et al. 1996).

Although the methods used to produce transgenic plants differ substantially from traditional breeding, it has been suggested that transformation should not affect rates or patterns of hybridization (Raybould and Gray 1994); therefore, information gathered on the movement of other crop markers provides information on the potential for movement of transgenes. It should, be noted however, that this will only be true when transgenes are located in chromosomal segments that are under similar selective regimes to the markers under consideration and if the transgenes themselves are neutral or favorable. This points to an important limitation of studies that consider only measures of F_1 crop-weed hybrid fitness as indicators of the potential for transgene spread (e.g., Langevin et al. 1990; Klinger and Ellstrand 1994; Frello et al 1995; Linder and Schmitt 1995; Arriola and Ellstrand 1997; Jørgensen et al. 1996; Mikkelsen et al. 1996; Snow et al. 1996). Even hybrids of fairly low fitness may successfully transmit transgenes to later generations of backcrossed progeny if the chromosomal segments carrying the transgenes do not impart a significant fitness cost to the hybrids (Gliddon 1994). Conversely, highly fit hybrids may be unlikely to transmit certain loci, including transgenes, to later generation hybrids, if these loci occur in chromosomal segments that are strongly negatively selected (Rieseberg et al. 1996). The fitness of later generation hybrids will provide a more accurate estimate of the likelihood of transgene persistence (Darmency 1994; Linder and Schmitt 1994); however, even these measures will not predict transgene movement in all cases. Therefore, inferences about transgene persistence from hybrid fitness measures, or from the movement of non-transgenic cultivar markers in hybrids, must be made with caution.

The crop markers that we have examined appear to behave as neutral or near-neutral alleles, because their frequencies fluctuated (Fig. 2) but did not increase or decrease over the five generations following hybridization (Fig. 3), and because the two markers did not differ significantly in their levels of introgression (Table 1; a coincidence that seems more likely under neutral conditions than if the markers were associated with positively or negatively selected traits.) Because of the close relationship between cultivated and wild sunflowers (Arias and Rieseberg 1995), it seems unlikely that chromosomal or genic sterility barriers will contribute to preventing the movement of cultivar alleles into wild *H. annuus*. Thus the movement of transgenes between cultivated *H. annuus* and wild sunflowers will depend largely on the fitness consequences of the transgene itself, rather than on its chromosomal location.

The practical consequences of transgene escape will depend on the nature of the engineered trait and its effect on wild and weedy populations (Ellstrand and Hoffman 1990; Dale 1994). Transgenic traits that are currently under development in sunflowers include oil characteristics, seed storage modification proteins, herbicide tolerance, biological control (i.e., *Bacillus thuriengensis*), tolerance to insect pests such as seed weevils, stem weevils, cutworms and sunflower beetles, tolerance to diseases including downy mildew, rust and verticillum wilt, and drought tolerance (Snow and Morán-Palma, 1997). The potential impact of spread of each group of characters should be carefully considered. Escape of transgenes affecting tolerance to control agents such as herbicides seems most likely to exacerbate weed control should these agents be needed to control weedy sunflowers. Transgenes that could interfere with factors that may limit growth of weedy or wild populations of *H. annuus*, such as tolerance to naturally occurring pests and diseases, may impact on the natural dynamics of these populations and potentially lead to ecological release. These effects are not easily predicted, and our data suggest that the potential impact of each transgene on weedy sunflowers should be carefully examined prior to commercial release because of the high probability of escape and persistence.

A further consideration surrounding the potential consequences of transgene escape in sunflowers is the possibility of gene transfer to other annual *Helianthus* species. *H. annuus* is known to hybridize with a number of annual members of the genus (Heiser et al. 1969; Rieseberg et al. 1988, 1990, 1991; Dorado et al. 1992). For example, *H. annuus* and *H. petiolaris* often form large hybrid swarms where they co-occur, and although hybrids have reduced fertility, introgression of *H. annuus* markers into *H. petiolaris* has been documented in a number of populations (Dorado et al. 1992).

We found no evidence that gene flow was occurring via achenes dispersing from the cultivar into weedy populations. If this is true for other populations, limiting transgene escape rests primarily on preventing pollen movement. Among the measures that have been proposed to decrease the likelihood of transgene escape via pollen flow are clearing wild relatives from areas immediately adjacent to cultivated fields, planting pollen-trap crops surrounding transgenic cultivars, and introducing genetic isolating mechanisms such as male sterility into transgenic cultivars (Ellstrand and Hoffman 1990; Raybould and Gray 1994). Previous work by Arias and Rieseberg showed that low levels of gene flow occurred up to 1000 m from the edge of cultivated

sunflower fields, and thus isolation by distance appears to be an impractical means of limiting hybridization (Kareiva et al. 1994). Our findings indicate that cultivar genes are capable of persistence in weedy populations, and thus even low levels of hybridization may result in transgene establishment in weedy sunflower populations. This finding suggests that the use of genetic isolating mechanisms, such as male sterility, is likely to be the most effective means of preventing the release of transgenes from cultivated sunflowers via hybridization.

Acknowledgments The completion of this project was greatly facilitated by technical assistance from Steven Cologne, John Gabel, John Gajewski, Ani Kheyrandish, Paula McMahon, Bianca Payne, Jeffrey Regan, and Guadalupe Valladares. We also greatly appreciate statistical help from Dick Repasky and Naoki Takabagoshi. This work was supported by USDA grant 92373007590 to L.H.R and A.A.S.

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