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Gene flow between cultivated and wild sunflowers

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Abstract With the development of transgenic crops, concern has been expressed regarding the possible escape of genetically-engineered genes via hybridization with wild relatives. This is a potential hazard for sunflowers because wild sunflowers occur as weeds in fields where cultivated sunflowers are grown and hybridization between them has been reported. In order to quantify the potential for gene escape, two experimental stands of sunflower cultivars were planted at two sites with different rainfall and altitude profiles. Populations of wild plants were planted at different distances from each cultivar stand. An allele homozygous in the cultivar (*6Pgd-3-a*), but absent in the wild populations, was used as a molecular marker to document the incidence and rate of gene escape from the cultivar into the wild populations of sunflowers. Three-thousand achenes were surveyed to determine the amount of gene flow from the cultivated to the wild populations. The marginal wild populations (3 m from the cultivar) showed the highest percentage (27%) of gene flow. Gene flow was found to decrease with distance; however, gene flow occurred up to distances of 1000 m from the source population. These data suggest that physical distance alone will be unlikely to prevent gene flow between cultivated and wild populations of sunflowers.

Key words Hybridization · Gene flow · Sunflowers Transgenes

Introduction

Plant breeders have long been concerned with gene flow between crop plants and their wild relatives (Anderson 1949; Harlan 1965; De Wet 1975; Barrett 1983; Ellstrand 1988). Early studies mainly focused on gene flow into crop strains because of fears that seed lots would be contaminated by foreign germplasm (Sprague 1938; Crane and Mather 1943; Haskell 1943; Bateman, 1947 a, b, c; Jones 1948; Hutchcroft 1955; Nieuwhof 1963). More recently, concern has been expressed regarding gene flow from crop plants into their wild relatives via hybridization. In particular, it has been suggested that genetically-engineered genes (transgenes) may be transferred into natural or weed populations through hybridization, potentially creating invasive weeds or increasing the difficulty of weed control (Ellstrand 1988, 1992; U. S. National Research Council 1989; Crawley 1990; Keeler and Turner 1990; Manasse 1992).

Nonetheless, there are few examples where the extent of gene flow between cultivated and weedy populations have been quantified (Kirkpatrick and Wilson 1988; Langevin et al. 1990; Klinger et al. 1991; Till-Bottraud et al. 1992; Wilson and Manhart 1993). The lack of quantitative data documenting crop-weed gene flow can be traced to a number of possible causes including the facts that: (1) the potential environmental consequences of crop-weed hybridization have only recently been widely publicized; (2) most of the earlier work employed characters whose genetic basis was unknown, making it difficult to determine whether the observed patterns of variation were due to hybridization and introgression, convergent evolution, or plasticity; (3) because of the close relationships between many crops and their wild relatives, it has often been difficult to find genetically-based markers that are exclusive to the crop plant.

The potential for gene exchange within the crop/weed complex depends on successful gene flow by pollen (Ellstrand and Hoffman 1990; Kareiva et al. 1991). Factors that increase the likelihood of gene exchange include self-incompatibility, high outcrossing rates, and generalist pollination mechanisms. Crop and weedy relatives must also have overlapping flowering periods, occur sympatrically, and be compatible (Keeler and Turner 1990). Many cultivated plants have weedy relatives growing along the field

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margins and some crops hybridize easily with their wild relatives. For example, hybridization between crop/wild populations has been suggested in rice (Oka and Chang 1959, 1961; Oka and Morishima 1971), sorghum (Dogett and Majisu 1968; Baker 1972), carrot (Small 1984), and pearl millet (Brunken et al. 1977). Thus, the movement of transgenes from crops into wild species is plausible, and hybridization has been suggested as the immediate hazard when genetically-engineered crops are released into the environment (Colwell et al. 1985; Goodman and Newell 1985; Tiedje et al. 1989).

The domesticated sunflower (*Helianthus annuus* L.) and its weedy relatives represent an appropriate experimental system for studying the potential for transgene escape and its consequences. Domesticated and weedy sunflowers grow side by side in many locations. They overlap in flowering time (late May through early October) and are visited by honeybees, bumblebees, and solitary bees. Wild *H. annuus* is self-incompatible, whereas the domesticated sunflower is self-compatible. The weedy *H. annuus* occurs in fields and along roadsides throughout the United States and Northern Mexico, whereas the domesticated sunflower is commonly cultivated in the plains states and California (McCormick et al. 1992). The domesticated sunflower is also widely cultivated in the Commonwealth of Independent States (the former Soviet Union), Argentina, Europe, and Mexico (Heiser 1976; Putt 1978).

Previous investigations have documented the occurrence of intraspecific and interspecific hybridization in *H. annuus* and its close relatives (Heiser 1954, 1976; Stebbins and Daly 1961; Heiser et al. 1969; Dedio and Putt 1980; Chandler et al. 1986; Rieseberg et al. 1988; Dorado et al. 1992; Rogers et al. 1982; Rieseberg and Brunsfeld 1992). Under artificial conditions the domesticated sunflower has produced fertile hybrids when crossed with wild *H. annuus*. Moreover, partially-fertile hybrids have been produced from wider crosses between the domesticated sunflower and other *Helianthus* species (Heiser et al. 1969). These studies suggest the possibility of gene flow from sunflower cultivars into natural populations, yet the frequency and rate of gene flow occurring between sunflower crops and their weedy relatives have not been documented.

Here, we report the frequency and rate of gene flow from domesticated sunflowers into weedy populations and show how these rates are influenced by distance. These results allow us to evaluate the utility of distance as an isolation barrier for experimental plantings of transgenic sunflowers.

Materials and methods

Experimental design

The cultivars were planted and maintained to simulate commercial plantings. Thus, honeybee hives were placed near the cultivar stands to enhance sunflower pollination. To estimate the utility of physical distance as a hybridization barrier we employed an experimental design similar to that used by Klinger et al. (1991). Two experimental stands of *H. annuus* were planted in early July 1992, one at the Universidad Autonoma del Estado de Morelos Biological Station in Cuernavaca, Morelos, Mexico, and the second one at the Agronomic Station in Cuautla, Morelos. The sites differ in rainfall, altitude, and soil type.

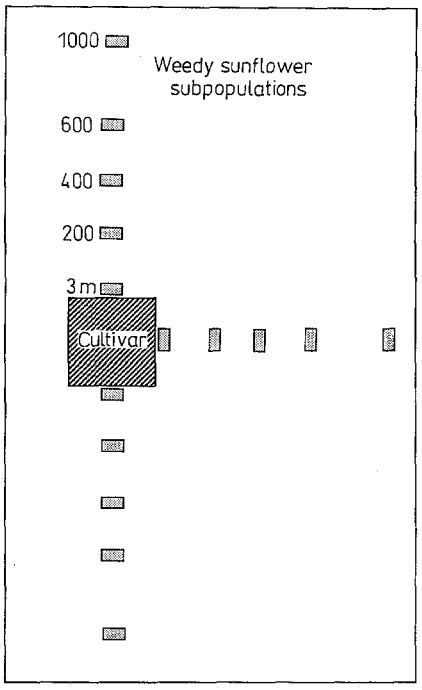

Fig. 1 Map of the experimental design showing the distribution of the weedy subpopulations around the sunflower cultivar stand. Population sizes and distances from cultivar are not to scale

Around each stand of domesticated sunflower, 15 subpopulations of ten individuals each of weedy *H. annuus* were established at distances of 3 m, 200 m, 400 m, 600 m, and 1000 m with three replicates at each distance (Fig. 1). These weedy populations of *H. annuus* were grown from achenes collected from natural populations. Achenes were germinated in Petri dishes at room temperature and, after 2 weeks, seedlings were transplanted and grown in a greenhouse for 4 weeks before they were transferred to the field. Each site was checked for naturally-occurring sunflower plants which were removed. The experimental stands were monitored until achenes reached maturity (approximately 14–16 weeks after germination) and were collected.

The sunflower cultivar (Peredovik'66) used in this experiment is a modern cultivar homozygous for allele *a* at the 6-phosphogluconate dehydrogenase enzyme locus (*6Pgd-3-a*, Rieseberg and Seiler 1990). This allele is rare in weedy populations. To ensure the absence of this specific molecular marker in the weedy populations, all individuals included in this experiment were surveyed for *6Pgd-3-a*, and individuals carrying the allele were discarded. A total of ten heads were harvested from each of the ten individuals of each replicate. The sunflower heads were dried at room temperature, and ten achenes from each head were selected randomly. Electrophoretic analysis of the achenes was used to assess the transfer of the molecular marker into the weedy populations.

Enzyme electrophoresis

Sample preparation and electrophoresis of 6PGD followed the sunflower isozyme protocol described by Rieseberg et al. (1988) and Rieseberg and Seiler (1990). Enzyme extraction was accomplished

by grinding leaves and achenes (after soaking for 24 h in distilled water) in a Tris-HCl-PVP grinding buffer (Soltis et al. 1983). Enzyme activity was resolved using 12.0% starch gels and buffer system 9 (Rieseberg and Seiler 1990).

Data analysis

Both the frequency and the rate of gene flow were estimated for each experimental stand. Frequency is defined as the proportion of hybrid progeny over total progeny at each distance (one-dimensional analysis) and is calculated on a per-target plant basis. Rate is defined as the total expected pollen flow at each distance if the population occurred as a concentric circle around the source populations and is calculated by multiplying hybrid frequencies by *(pi) d* (diameter) (Grosberg 1991; Kareiva et al. 1991; Klinger et al. 1991, 1992). This value is useful for extrapolating from experimental populations, such as the ones reported here, to natural populations of wild sunflowers which often completely encircle cultivar fields.

The effects of distance on frequency and rate of hybridization at each site were analyzed with a one-way analysis of variance (ANOVA, SYSTAT, 1992). Differences between sites were tested using a two-way ANOVA. Frequency values used in both the one-way and two-way ANOVA were arcsine square-root transformed in order to prevent values close to zero from having constrained variances.

Results

The incidence of crop/weed hybridization in sunflowers varied greatly among plants and populations. Overall, 10% (299 of 3000) of the seeds analyzed were hybrid individuals. Frequency values per plant ranged from 0 to 0.60. Pollen dispersal was found to be inversely proportional to distance from the pollen source (Fig. 2 a, b). All plants surveyed at 3 m had some hybrid progeny and mean hybrid frequency was 0.27; however, frequency declined to 0.15 at distances of 200 m, and to less than 0.05 at distances of 400 m or more. A hybrid frequency of 0.02 was detected for one site at 1000 m (Fig. 2 a). However, no gene flow was detected at 1000 m at the second site (Fig. 2 b). The observed differences in frequency between distances were significant for each site ($P<0.001$).

Comparison of the frequency of gene flow between the two sites revealed no significant differences ($P=0.057$). Nonetheless, there does appear to be a trend toward lower gene flow levels at 400–1000 m at the Cuautla site (Fig. 3). In general, the further the distance from the source plant, the wider the difference between mean frequency values at the two sites.

In contrast to the one-dimensional analysis, rates of total pollen dispersal were greater at distances of 200 to 1000 m than along the crop margin (Fig. 4). That is, if the weed population occurred as a concentric circle around the cultivar field, the relative amount of pollen required to produce the observed hybridization frequencies is greater at distances above 200 than at 3 m. As with the frequency data, differences in gene-flow rates between distances were significant for each site (Cuernavaca, $P< 0.003$; Cuautla, $P< 0.001$). In contrast to the frequency data, however, significant differences were observed in gene-flow rates between sites. For example, at the Cuernavaca site the highest amount of pollen dispersal was at distances of 400 and 600 m (Fig. 4), whereas Cuautla had its highest rate at a distance of 200 m (Fig. 4). As observed for the frequency data, the further the distance from the cultivar stand, the wider the difference between the mean-rate values at each site.

Discussion

This study shows that gene flow can occur between cultivated and weedy *H. annuus* populations. Although the frequency of hybrid progeny varied dramatically among the weedy subpopulations (0–37%), the overall mean frequency values show that one out of every ten achenes surveyed in the study is of hybrid origin. Similar results have been observed for other crop-weed complexes. For example, analysis of hybridization between cultivated- and red-rice revealed a frequency of hybrid seed set ranging from 1 to 52% (Langevin et al. 1990). Likewise, in an experi-

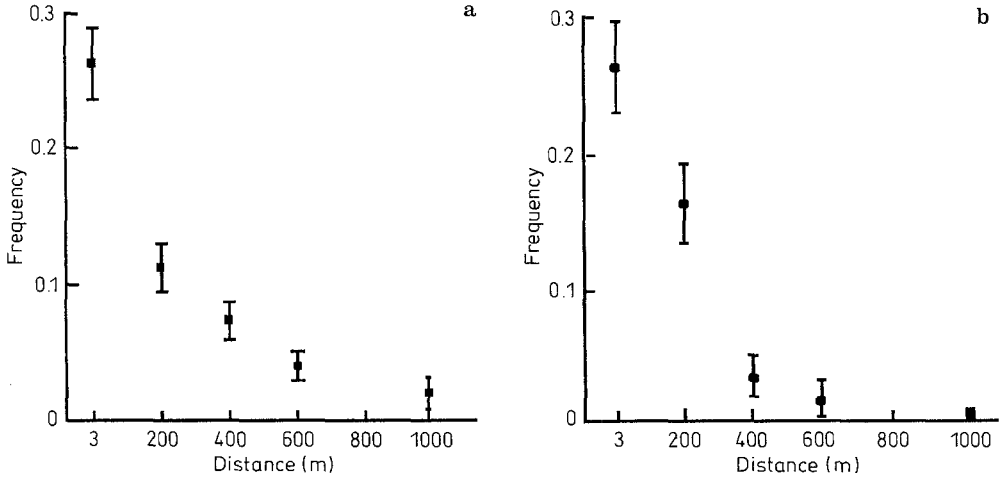

Fig. 2 a, b Frequency of sunflower cultivar marker among progeny of weed populations at the five distances. Values represent the mean and SE at each distance (n=30). **a** Cuernavaca site; **b** Cuautla site

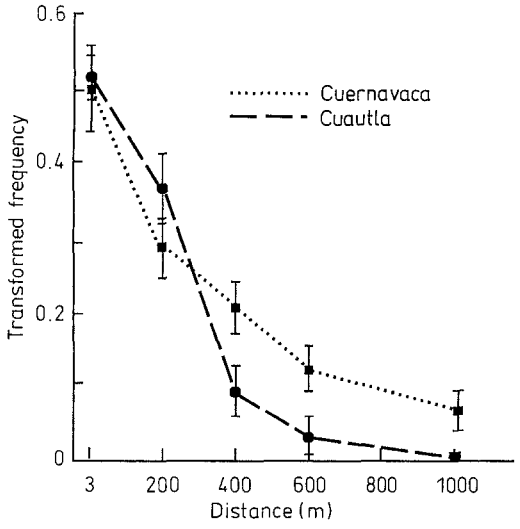

Fig. 3 Transformed frequency of sunflower cultivar marker among the progeny of weed populations at the five distances and two sites

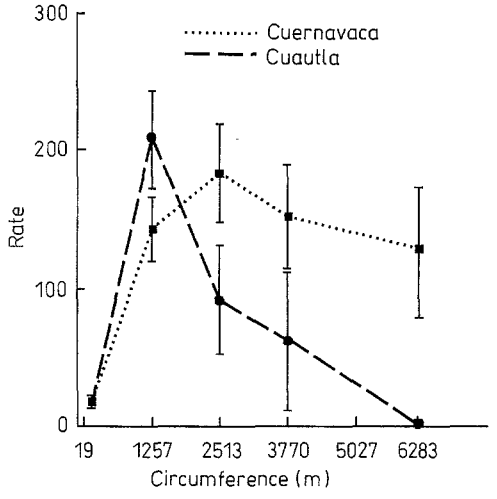

Fig. 4 Estimated rate of pollen dispersal from the sunflower cultivar into the weed populations at the five distances and two sites. Values represent the mean and SE at each distance (n=30)

ment similar to that presented here, Klinger et al. (1991, 1992) demonstrated gene flow levels ranging from 1 to 40% between cultivated and wild radish populations.

The data presented in the present study also demonstrate that pollen can travel surprisingly large distances in sunflowers. Hybrid progeny (2–7%) can be expected at 800 m and sometimes as far as 1000 m (2%). Other studies in crop/weed complexes which have employed molecular markers also provide evidence for long-distance pollen dispersal. Klinger et al. (1991) observed 1% gene flow at a distance of 1000 m in radish. Kirkpatrick and Wilson (1988) detected an allele specific to cultivated *Cucurbita pepo* in 5% of the progeny of the native *Cucurbita texensis* at a distance of 1300 m.

As previously mentioned, sunflowers possess numerous characteristics that can increase the risk of gene escape. Cultivated sunflowers are grown in areas where wild *H. annuus*, *H. bolanderi*, *H. petiolaris*, *H. debilis*, and *H. niveus* also occur. Cultivated *H. annuus* overlaps in flowering period and hybridizes to some extent with all the taxa mentioned above (Rogers et al. 1982). Concern must be raised not only in areas where other wild sunflower species grow, but also where wild taxa have been introduced in areas where *H. annuus* is cultivated. For example, wild *H. annuus* has been introduced throughout much of the world and occurs as a weed in all areas where sunflower is cultivated.

Ecological and genetic variables, such as pollinator behavior, abundance of weed plants and flowering patterns, may also affect the frequency and rate of gene flow (Bateman 1947a, b, c). In the present experiment, a significant difference was observed between the two sites in terms of gene-flow rates, with higher rates of hybridization at greater distances in the Cuernavaca site versus the Cuautla site. One possible explanation is the different ecological conditions present in the two sites. Cuautla is characterized by a shorter rainy season, less rainfall, and a higher temperature than Cuernavaca. Possibly, the higher temperatures at Cuautla influence the life span of pollen at this site, potentially reducing the viability of pollen transported over long distances. Differences in species richness or abundance, and the constancy of wild bees, may also influence pollen dispersal. These factors have been shown to affect achene production in cultivated sunflowers (Free 1964). Other possible explanations include differences in the composition of plants bordering the sites and flowering phenologies. Nonetheless, the data suggest the need to consider both biotic and abiotic factors influencing pollen dispersal and hybridization.

Generally, physical distance is the method employed to prevent gene escape between a crop and its compatible relatives (Sprague 1938; Haskell 1943; Crane and Mather 1943; Bateman 1947 c; Jones 1948; Hutchcroft 1955; Nieuwhof 1963). However, in sunflower the distance required for isolation must be greater than 1000 m, and this isolating barrier cannot be considered impermeable. Distance may be an effective isolating barrier for research purposes. Nonetheless, as the uses of transgenic plants increase, the certainty of containment may decrease. It has also been suggested that border rows of nontransgeneic cultivars be established to intercept escaping pollen. This precaution may be effective for small field trials, but as the commercial use of transgenic crops increases, more stringent safeguards may be required. Methods of inducing genetic isolation through male sterility, chromosomal sterility, aneuploidy and polyploidy, must also be considered (Keeler and Turner 1990). For example, *H. annuus* is known to be differentiated from all of its close relatives by a partial chromosomal sterility barrier, and it is possible that some combination of physical distance and chromosomal incompatibility could serve as a reasonably effective, although not impermeable, barrier to gene exchange.

To better understand the process of crop-weed hybridization in sunflowers, future work should focus on the effects of cultivar genotype, size and shape of source and re-

cipient populations, pollen competition, and hybrid fitness, on gene-flow rates. These results, combined with the information presented here, should be directly applicable to regulatory guidelines concerning the isolation of transgenic sunflower cultivars and other outcrossing, insect-pollinated crop species.

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