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Genomic map of a diploid hybrid species

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Several fertile diploid plant species have been shown to be derived from hybridization between species with strong chromosomal sterility barriers, yet little is known about the genomic processes that accompany this mode of speciation. As a first step toward understanding these processes we have generated a detailed genetic linkage map for *Helianthus anomalus*, a diploid species derived via hybridization between *H. annuus* and *H. petiolaris*. This was generated using 161 RAPD loci and one isozyme locus. The genetic markers were distributed into 18 linkage groups and cover 2338 cM. Analysis of the parental origin of each locus/allele revealed that 44 were originally derived from *H. annuus*, 37 were derived from *H. petiolaris*, 54 could have been derived from either parent, and 25 were unique to *H. anomalus*. In addition, the parental linkage groups were not transmitted or retained intact in the hybrid species. Rather, molecular markers originating from both parental species were interspersed within individual *H. anomalus* linkage groups. Although there are several limitations to analysing hybrid genomic composition using this approach, these results do raise the possibility that diploid hybrid species may be able to retain different portions and/or proportions of their parental species' genomes. This characteristic may provide diploid hybrid species with greater flexibility than allopolyploid species in terms of optimizing their genomes for a new ecological niche and may permit the production of several different diploid hybrid species from the same two parents, as appears to have happened in *Helianthus*.

Keywords: genomic mapping, *Helianthus*, hybrid speciation, RAPDs, sunflowers.

Introduction

A major impediment to the origin of new species via hybridization is the partial chromosomal sterility characteristic of first-generation hybrids between chromosomally differentiated parents. In many organisms, allopolyploidy provides the means to circumvent hybrid sterility — full fertility is achieved immediately following genome doubling. Alternatively, several authors have proposed genetic models whereby the impasse of chromosomal sterility is bypassed without a change in ploidal level (Grant, 1958; Stebbins, 1957). This model of speciation, termed recombinational speciation by Grant (1966a), has been verified by the artificial synthesis of new diploid hybrid species in several plant genera (Grant, 1966a, b; Smith & Daly, 1959; Gerassimova, 1939; Stebbins, 1957). The results from the theoretical and experimental studies indicate that recombinational speciation is a workable process under artificial conditions and may therefore occur in nature. Nevertheless, two significant questions remain to be addressed: (i) what is the extent of recombinational speciation in nature? and (ii) what genomic processes accompany or facilitate this mode of speciation?

For the past several years, we have focused on the first question using the annual sunflowers of the genus *Helianthus* as a model group. We have demonstrated that at least three and possibly as many as four of the 11 species comprising *Helianthus* section *Helianthus* are stabilized hybrid derivatives (Rieseberg *et al.*, 1990b; Rieseberg, 1991a). Furthermore, hybridization has played a significant role in the evolution of several geographical races (Rieseberg, 1991a; Rieseberg *et al.*, 1990b, 1991a, b; Dorado *et al.*, 1992). Thus, recombinational speciation is significant in this group.

The goal of the present paper is to begin to address the second question — what genomic processes occur during recombinational speciation? As a first step toward this goal, we have generated a detailed genetic linkage map for one of the four diploid hybrid sunflower species, *H. anomalus* Blake, using 161 random amplified polymorphic DNA (RAPD) loci and one isozyme locus. We have also surveyed natural populations of both parental species, *H. annuus* L. and *H. petiolaris* Nutt., for those alleles and loci mapped in *H. anomalus* in order to document the origin and distribution of parental molecular markers in the *H. anomalus* nuclear genome. Here we present the genomic map for *H.*

anomalus resulting from this work and address the following specific questions.

1 How are the parental genomes recombined in the hybrid species, *H. anomalus*? Are there recognizable parental 'subgenomes' (i.e. one group of chromosomes corresponding to one parent and a second group corresponding to the second parent) or are molecular markers of both parents interspersed within chromosomes?

2 Is the contribution of each parental genome to the hybrid species roughly equivalent or are the genomes of the hybrid species strongly biased toward one parent or the other? For example, did *H. petiolaris* contribute a greater portion of its genome to *H. anomalus* than did *H. annuus* as *H. petiolaris* is more similar to *H. anomalus* in terms of ecological preference (below)?

In addition, we discuss the advantages and limitations of RAPD loci for genomic map construction in wild plant species and suggest several approaches for increasing the utility of this new class of molecular markers.

The three sunflower species used to address these questions are obligately outcrossing annuals with the same chromosome number (Heiser *et al.*, 1969). The two parents, *H. annuus* and *H. petiolaris*, are widespread, polytypic species that are easily distinguished by a number of morphological, chromosomal, and molecular features (Heiser *et al.*, 1969; Schilling & Heiser, 1981; Chandler *et al.*, 1986; Rieseberg, 1991a; Rieseberg *et al.*, 1991a). The two species occur in divergent clades based on chloroplast DNA (Rieseberg *et al.*, 1991a), nuclear ribosomal DNA (Rieseberg, 1991a), and morphological analyses (Schilling & Heiser, 1981), and each species has different ecological requirements. In general, *H. annuus* occurs in heavy soils, whereas *H. petiolaris* is restricted to dry, sandy soils. Nevertheless, the two species grow together in a variety of locations, and hybrid swarms are common. Artificial hybridization experiments have revealed that the two species are different chromosomally (Chandler, *et al.*, 1986; Ferriera, 1980; Heiser, 1947), with a wide range of multivalent configurations observed at meiosis that apparently correspond to several translocations and inversions. Pollen viabilities in artificially synthesized F_1 hybrids range from 0 to 30 per cent, and seed set is less than 1 per cent (Heiser, 1947; Chandler *et al.*, 1986). The percentages of good pollen in F_2 plants are highly variable, ranging from 13 to 97 per cent, suggesting that it may be possible to overcome the sterility barrier in the F_2 and later generations (Heiser, 1947), a prerequisite for recombinational speciation.

Helianthus anomalus, in contrast, is a rare, xerically adapted sunflower endemic to sand dune and swale habitats in northern Arizona and southern Utah (Heiser, 1958; Nabhan & Reichhardt, 1983). Morphologically it is quite distinct from its parent due to its long, narrow phyllaries and its pronounced tuberculate hairs on leaves, phyllaries, and stem. However, molecular data provide unequivocal evidence for its hybrid origin in that it combines the ribosomal DNA repeat units and isozymes alleles of *H. annuus* and *H. petiolaris* (Rieseberg, 1991a). Furthermore, individuals of the species possess either the chloroplast DNA genotype of *H. annuus* or *H. petiolaris* rather than a unique one, although the *H. annuus* plastome type is much more common than that of *H. petiolaris* (Rieseberg, 1991a). Artificial hybridization experiments indicate that *H. anomalus* is now reproductively isolated from its parents by a relatively strong chromosomal sterility barrier (Heiser, 1958; Heiser *et al.*, 1969; Chandler *et al.*, 1986). Pollen stainabilities in F_1 hybrids of *H. anomalus* × *H. annuus* ranged from 1.8 to 4.1 per cent (Heiser, 1958; Chandler *et al.*, 1986), whereas those for hybrids of *H. anomalus* × *H. petiolaris* ranged from 2 to 58.4 per cent (Heiser, 1958; Heiser *et al.*, 1969; Chandler, *et al.*, 1986). Chandler *et al.* (1986) suggest that *H. anomalus* is polymorphic for a translocation, which may account for the great variability in pollen stainability observed for hybrids with *H. petiolaris*.

Materials and methods

Plant materials

Selection of *H. anomalus* populations for the genomic mapping experiment was based on isozyme surveys of natural populations (Rieseberg *et al.*, 1991a; L. H. Rieseberg, unpublished observations). Populations that were most divergent isozymically within the species (*ANO-1497* and *ANO-1506*; locality data in Rieseberg, 1991a) were used as parents. The *H. anomalus* map was generated using 56 individuals derived from the intraspecific hybrid between these two populations crossed to an inbred sunflower line (CMS89). Thus, segregation of *H. anomalus* chromosomes could be monitored. This particular crossing design was used because of the need to map dominant RAPD loci (Williams *et al.*, 1990) and the difficulty of producing F_2s or backcrosses in wild, self-incompatible annuals.

The origins of the mapped *H. anomalus* alleles were determined by surveying two natural populations from each parental species: *H. annuus* (*A3* and *A21*; locality data in Rieseberg *et al.*, 1988), and *H. petiolaris* (*Rieseberg 1106* and *Reiseberg 1224*; locality data in Beckstrom-Sternberg *et al.*, 1991). The two *H. petiolaris* populations represent the two subspecies of *H. petiolaris*, ssp. *fallax* and ssp. *petiolaris*, respectively,

whereas the two *H. annuus* populations simply represent different portions of the geographical range of this species, being derived from California and Missouri, respectively. For allelic surveys, 10 individuals were assayed from each population, except for *Rieseberg 1106*, where seven plants were examined. RAPD analysis of two representative populations per parental species is justified because RAPD surveys of five populations of *H. annuus* and eight populations of *H. petiolaris* for five RAPD primers (62 and 58 putative loci, respectively) have revealed that most RAPD variation occurs among individuals within local populations: 82 per cent for *H. annuus* and 86 per cent for *H. petiolaris* (L. H. Rieseberg *et al.*, unpublished data). Furthermore, it should be stressed that we are interested in determining the 'most likely' parental contributor for each allele. Thus, if an allele is found in high frequency in one parent, but occurs so rarely as to not be sampled in the second parent, the likelihood that the second parent served as the allelic donor is minimal.

DNA isolations

One gram of fresh leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. The frozen powdered tissue was mixed with a CTAB extraction buffer (Doyle & Doyle, 1987), modified by the addition of sodium metabisulfate (1 per cent w/v). The resulting solution was filtered through a layer of miracloth, and DNA was extracted following the method of Doyle & Doyle (1987), except that a second chloroform extraction was performed. The purified DNAs were dissolved in TE and quantified on a fluorometer.

PCR primers, reaction conditions and gel electrophoresis

Two hundred and eighty arbitrary 10-mer oligodeoxynucleotide primers were obtained from the University of British Columbia Biotechnology Laboratory (primers 100–300) and Operon Technologies (primer kits A–D) and were tested for amplification products using two *H. anomalus* DNAs. Strong PCR products were produced by 160 (57 per cent) of these primers. Six segregating progeny were then screened for the 160 potentially useful primers to search for polymorphic PCR products. Seventy-four primers yielded polymorphic PCR products and were used to amplify purified genomic DNA samples from the 56 *H. anomalus* progeny, as well as from the 37 individuals from natural populations of *H. annuus* and *H. petiolaris* (primer sequence information is available upon request from the author). Amplification reactions and cycle parameters followed Williams *et al.* (1990). Amplification products were separated by electrophoresis on 1.5 per cent agarose gels and detected by staining with ethidium bromide (Fig. 1a).

Isozyme survey

Ten progeny of *H. anomalus* were screened for allozymic variation for the following enzymes: acid phosphatase, aconitate hydratase, alcohol dehydrogenase, aminopeptidase, aspartate aminotransferase, esterase, glucose-6-phosphate isomerase, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, peptidase, peroxidase, phosphoglucomutase, phosphogluconate dehydrogenase, ribulose-bisphosphate carboxylase, shikimate dehydrogenase, superoxide dismutase, and triose-phosphate isomerase. Sunflower isozyme protocols have been described previously (Rieseberg *et al.*, 1988; Rieseberg & Seiler, 1990). Variation was detected and scored for acid phosphatase (ACPH) only.

Data analysis

For each segregating locus, goodness of fit to a 1:1 ratio was determined by Chi-square analysis using the computer program LINKAGE-1 (Suiter *et al.*, 1983). Recombination fractions between all pairs of markers, linear gene order, and map distances were all determined by the computer program MAPMAKER (Lander *et al.*, 1987). The map was developed using a LOD score of 3.0 and a recombination limit of 0.33. The latter value represents the maximum recombination fraction at which linkage can be detected, as the 99 per cent confidence level, between markers utilizing 56 backcross progeny (Tanksley *et al.*, 1988b). The map of each ordered linkage group was then generated by the MAP function of MAPMAKER (Fig. 2).

When two amplification products from a single primer mapped to the same chromosomal location, co-dominance was hypothesized. In order to verify co-dominance, amplification products were transferred to Bio Trace (Gelman) nylon filters. The nylon filters were then hybridized to gel-isolated (Elu-Quick; Schleicher & Schuell), digoxigenin-labelled (Boehringer Mannhein) putative co-dominant products (Fig. 1b). Hybridization protocols followed Rieseberg (1991a).

The origin of the segregating allele(s) for each mapped locus in *H. anomalus* was determined by screening individuals from natural populations of *H. annuus* and *H. petiolaris* as described above and the

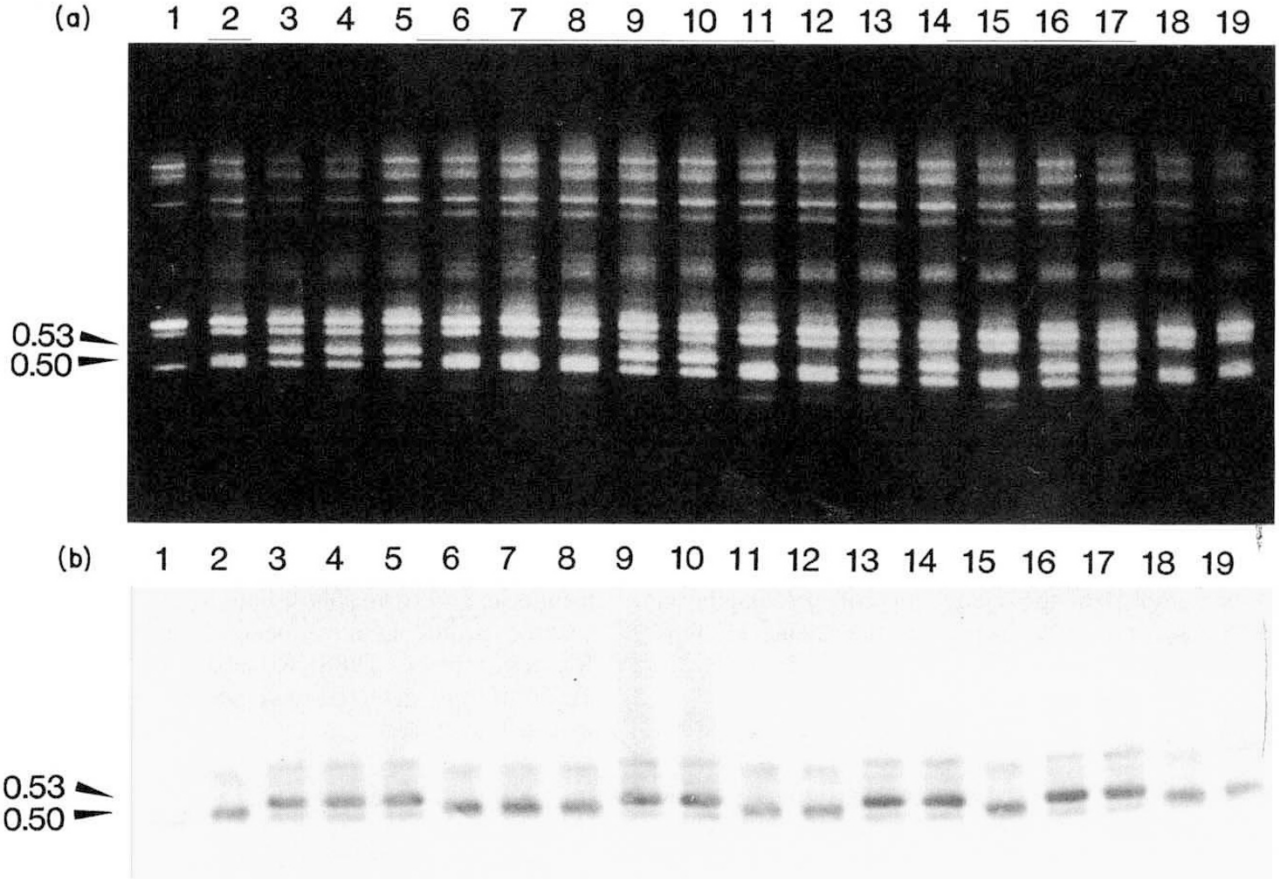

Fig. 1 (a) Segregation of an amplification polymorphism for primer 104 (Fig. 2) in the *H. anomalus* mapping population. Lanes 1, 2, and 3 are amplification profiles of the parents, CMS89, *ANO-1497*, and *ANO-1506*, respectively; lanes 4–19 are progeny. Two segregating polymorphic fragments are shown (0.50 and 0.53-kb, respectively). The 0.50-kb fragment is amplified in lanes 2, 6, 7, 8, 11, 12, 15, 18 and 19, whereas the 0.53-kb fragment is amplified in lanes 3, 4, 5, 9, 10, 13, 14, 16, and 17. (b) The 0.53-kb fragment was used as a hybridization probe to determine whether the two segregating fragments represent co-dominant alleles at a single locus. Both fragments hybridized, verifying co-dominance.

information manually plotted onto each linkage group to determine the genomic distribution of the parental molecular markers.

Results

RAPD markers

One hundred and sixty-one RAPD loci and one isozyme locus (ACPH) were scored for the 56 segregating progeny of *H. anomalus*. To assess whether the amplification patterns and subsequent genotypic scoring were reproducible, we re-amplified and rescored the entire progeny array of seven primers (15 loci). Eight discrepancies were observed between the 840 original and rescored genotypes, suggesting an error rate of slightly less than 0.5 per cent. All discrepancies observed appeared to have resulted from incomplete amplifications (e.g. Fig. 1) and were only observed for segregating fragments greater than 1.1 kb in size. These observations do not include faint bands, which were not reliably amplified from run to run and were not scored.

Eleven (6.8 per cent) of the 161 RAPD loci, as well as ACPH, were co-dominant (Figs 1 and 2). The remaining 150 RAPD loci were scored as dominant (i.e. presence versus absence of amplified fragments). For several loci, both parental alleles appeared to be amplified, but one of them could not be easily scored because it was faint or overlapped with other fragments, so these loci were scored as dominant.

Distorted segregation ratios were observed for 22 (13.6 per cent) loci (Fig. 2). These loci were generally clustered in specific regions of the genome, and the bias was for one or the other parental type within each group. This is not an unusual observation for genomic mapping studies (e.g. Bonierbale *et al.*, 1988; McCouch *et al.*, 1988; Gebhardt *et al.*, 1989; Havey & Muelh-

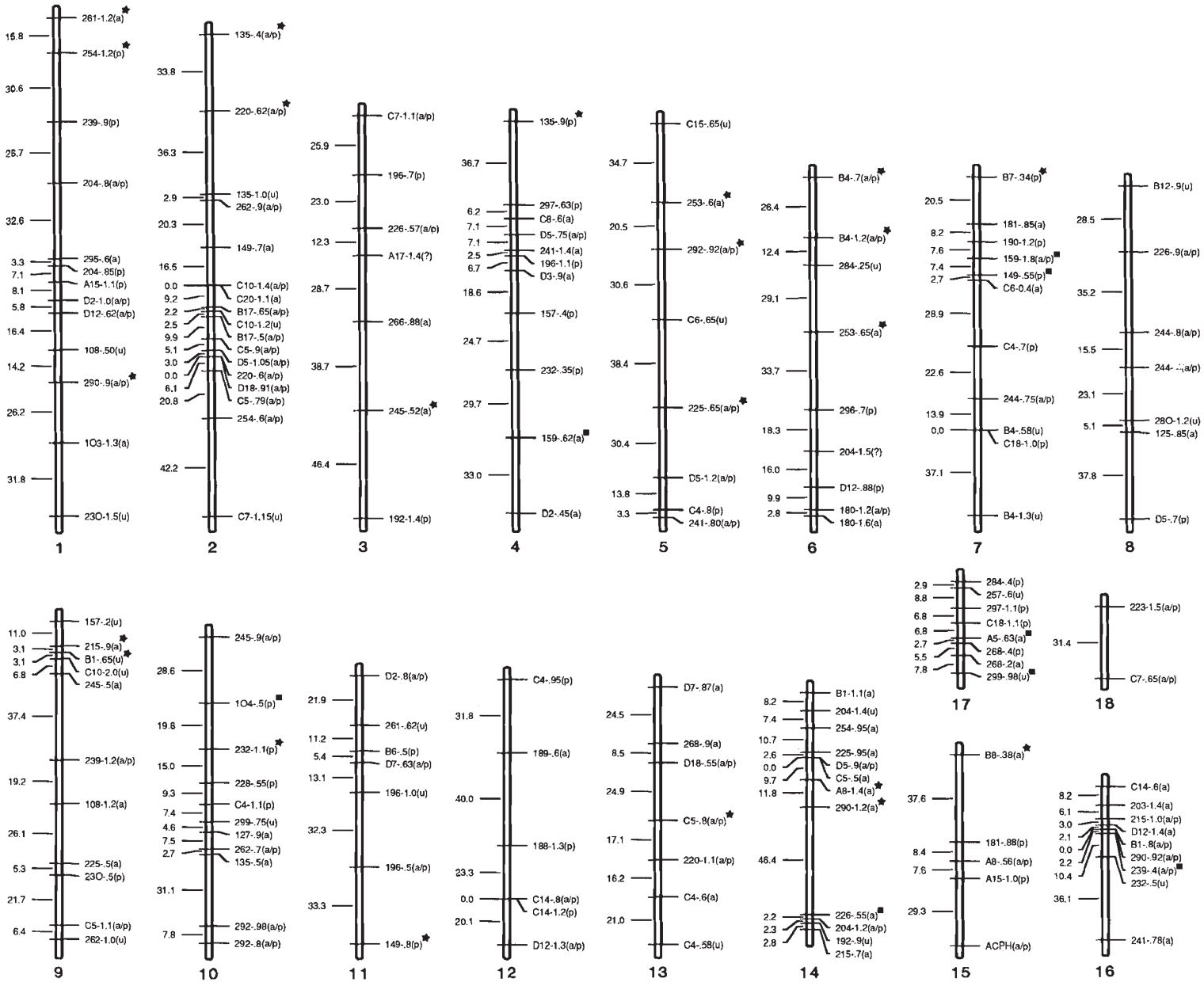

Fig. 2 Genetic linkage map for *H. anomalus*. The 18 linkage groups are listed at the bottom. Map distances in centiMorgans are listed on the left and loci on the right of each linkage group. Locus nomenclature includes, from left to right, the primer designation and the size in kilobases of the segregating allele scored. For co-dominant alleles (indicated by a square), only one of the two alleles is given (the other alleles are as follows: 295–0.55, 159–0.58, 180–1.10, 159–1.50, 149–0.40, 104–0.53, 261–0.55, 226–0.53, 239–0.65, A5–1.30, 299–0.97). The letters in parentheses following each locus/allele indicate its parental origin: a = *H. annuus*, p = *H. petiolaris*, a/p = *H.annuus* or *H. petiolaris*, and u = unique to *H. anomalus*. The star indicates a distorted segregation ratio. Molecular marker 190–1.1(a) was unlinked. The primer sequence information is available upon request from the authors.

bauer, 1989; Slocum *et al.*, 1990; Landry *et al.*, 1991), and simply suggests that RAPD loci or linked sequences can be under positive or negative selection.

Genomic map

The 162 polymorphic loci were mapped to 18 linkage groups (Fig. 2) and were assigned numerical designations from 1 to 18, based on their size in centiMorgans (if the recombination limit was raised to 0.35 [95 per cent confidence limit for 56 backcross progeny], the 162 loci mapped to 17 linkage groups, corresponding to the haploid chromosome number of *H. anomalus*). The total map distance of the *H. anomalus* linkage groups was 2338 cM, with an average distance of 16.4 cM between adjacent loci. This represents one of the largest plant genomes mapped to date. Although all but one locus could be linked significantly (LOD 3.0), several loose linkages were observed at the ends of linkage groups 2, 3, and 14. In these instances, both the proposed linkages and accompanying map distances should simply be considered as best estimates until additional loci are mapped.

Surveys of natural populations of *H. annuus* and *H. petiolaris* (Fig. 2) revealed that 27 per cent (44/162) of the loci appear to be derived from *H. annuus* and 23 per cent (37/162) from *H. petiolaris*. The parentage of the remaining 81 loci could not be determined because the allele(s) found in *H. anomalus* occurred in both parental species (33 per cent; 54/162), were unique to *H. anomalus* (15 per cent; 25/162), or could not be scored in the parental populations (1 per cent; 2/162). For the loci of known parental origin, 54 per cent (44/81) were derived from *H. annuus* and 46 per cent (37/81) from *H. petiolaris*. A chi-square test revealed that these proportions did not differ significantly from the expected 1:1 ratio.

Loci of *H. annuus* and *H. petiolaris* were interspersed for 13 linkage groups, indicative of extensive recombination between the parental species during the initial hybridization event. Loci from one parent or the other were missing for the remaining five linkage groups (2, 13, 14, 15, 16). However, many loci of unknown parentage mapped to these linkage groups, and it seems likely that if parentage could be determined for these loci, or if additional loci were mapped, that a recombinant pattern would be observed for some of these linkages as well.

Discussion

One of the major goals of evolutionary biology is to understand those genetic processes accompanying, facilitating, or resulting from different modes of speciation. Detailed knowledge of these processes may lead to more reliable predictions concerning the likelihood of occurrence or extent of different modes of speciation in nature. Likewise, the study of genetic processes may lead to the discovery of particular genetic consequences unique to a mode of speciation, which may then be used for diagnostic purposes in future biosystematic studies. Finally, these types of study may yield new insights into the evolutionary potential of a particular type of speciation; i.e. what is the likelihood that a new entity originating via this process will actually found major new evolutionary lineages?

The ability to generate saturated genetic linkage maps from a single segregating progeny has resulted in a new approach to the study of genetic processes. Comparison of genomic maps between closely (Bonierbale *et al.*, 1988) and more distantly related species (Tanksley *et al.*, 1988a) in the Solanaceae has provided unique insights regarding the conservation (or lack thereof) of gene order and gene repertoire in this family. A similar approach has been employed to assess the extent and distribution of duplicated sequences in both diploid (Song *et al.*, 1988, 1991) and putative ancient polyploid species (Helentjaris *et al.*, 1988). Nonetheless, this approach has yet to be employed to examine the genomic consequences of diploid hybrid speciation or, for that matter, to examine any mode of speciation in plants. This may be due to the concentration of most workers' research efforts on the genetics of domesticated plant species. Alternatively, the time and expense of the traditional Southern hybridization approach may be prohibitive for most comparative studies of genome evolution.

The use of RAPD markers provides an efficient, reproducible, and relatively inexpensive approach to comparative genomic mapping studies (Williams *et al.*, 1990). In particular, the level of variation for RAPDs appears to be much higher than most comparable classes of molecular markers. For example, in this study only one out of 20 (5 per cent) enzymes exhibited variation, whereas 74 out of 280 (26 per cent) RAPD primers produced polymorphic amplification products for the same progeny array. However, RAPD variation does appear to be partitioned within and among sunflower populations in much the same manner as isozyme variation (Rieseberg *et al.*, 1991a), with the majority of diversity found within rather than among populations.

There are also a number of drawbacks to the use of RAPDs for genomic mapping including: (i) testcross or backcross families, which are necessary for the efficient mapping of dominant RAPD loci, may be difficult to obtain for many wild species; (ii) the assessment of

locus homologies among maps of related species may be problematic because several loci can be amplified by a single primer; and (iii) due to variation in mapping populations and thermocyclers used in different laboratories, results from different laboratories may not be completely comparable. Potential solutions to these problems include mapping haploid families derived from tissue cultured pollen, the use of Southern hybridizations to test locus homologies among maps, the development of co-dominant RAPD markers via restriction endonuclease digestions, and the use of longer primers in order to increase the comparability of data from different laboratories and thermocyclers.

This paper reports the first application of genomic mapping to the study of diploid hybrid speciation and describes one of the first genomic maps generated for a wild plant species using either RAPD or RFLP technologies. In addition, this study represents the first attempt to determine the genomic composition of a diploid hybrid species. Nonetheless, there are several limitations to analysing hybrid genomic composition using this approach. For example, because the parentage of many loci cannot be determined, it may not be appropriate to construct a 'graphical genotype' (Young & Tanksley, 1989) for the hybrid species. Thus, it may be that only the parental origin of particular loci, rather than actual chromosomal fragments, can be accurately determined. Moreover, the approach employed here is dependent on prior unambiguous documentation of diploid hybrid speciation (as is the case for *H. anomalus*) because any time three species share a recent common ancestor some interspersion of alleles is expected. Thus, this approach cannot be used to diagnose diploid hybrid species in nature. Finally, due to the limited number of parental populations surveyed, it is possible that some of the loci/alleles ascribed to one parent actually occur in the other parent as well, but were not detected in our survey.

Nevertheless, the genetic linkage map described here for *H. anomalus* does allow several insights into the genomic processes accompanying diploid hybrid speciation in sunflowers. First, the genomic data indicate that the parental linkage groups (*H. annuus* and *H. petiolaris*) are not transmitted or maintained intact as 'subgenomes'. Instead, the molecular markers of both parental species are generally interspersed within individual *H. anomalus* linkage groups. This was not the case, however, for five linkage groups (2, 13, 14, 15, and 16; Fig. 2), which lack loci/alleles from either one or the other parent. It seems likely, however, that if additional loci were mapped that a recombinant pattern would be observed for these linkages as well. Alternatively, some of these linkage groups may have been transmitted largely intact from the parental species. This would be theoretically possible for linkage groups that differ by a major rearrangement in the parental species, thus potentially limiting or even preventing recombination following the initial hybridization event. Note that *H. annuus* and *H. petiolaris* are known to differ by at least five translocations and inversions (Heiser, 1947; Chandler *et al.*, 1986).

A second insight from the genomic map concerns the relative genomic contributions of the parental species to the *H. anomalus* genome. Although we had initially predicted that *H. petiolaris* would be the major genomic donor to *H. anomalus*, this was not the case. Rather, the parental species contributions were roughly equivalent (54 per cent *annuus* to 46 per cent *petiolaris*) for those loci/alleles whose parentage could be determined.

Although the genomic contributions of *H. annuus* and *H. petiolaris* to the *H. anomalus* genome were not significantly different, the pattern observed does suggest the possibility that parental genomes are not necessarily transmitted or retained in equivalent amounts in diploid hybrid species. Consideration of this possibility may be important in assessing the potential role of this mode of speciation in nature. Grant (1966a, b) has suggested that recombinational speciation involving strong sterility barriers is rare relative to allopolyploid speciation due to 'hybrid breakdown' in diploid hybrid species. Hybrid breakdown refers to the tendency of many second and later generation hybrids to be less vigorous than their parents or even inviable, apparently due to 'disharmonious genic and chromosomal combinations' (Grant, 1963). Although hybrid breakdown may indeed represent a severe problem for diploid hybrids in certain plant groups, the ability of a diploid hybrid species to retain different portions or proportions of the parental genomes may allow them much greater flexibility in optimizing their genome for a specific ecological niche than an allopolyploid. Likewise, a single pair of parental species has the potential to give rise to several different diploid hybrid species, which are likely to be reproductively isolated both from each other and from their parents. This appears to be the case for *H. annuus* and *H. petiolaris*, which have given rise to as many as four new diploid hybrid species that differ from each other and their parents in terms of morphology, ecological preference, and secondary chemistry and which are reproductively isolated by chromosomal sterility barriers (Rieseberg, 1991a). In contrast, multiple origins of an allopolyploid species from the same two parents generally appear to lead to the formation of ecologically similar forms that are often indistinguishable by traditional systematic means

and lack isolating barriers (reviewed in Soltis *et al.*, 1992).

Grant (1966a, b) emphasizes that diploid hybrid species, unlike allopolyploid species, are free from restrictions on recombination and thus more likely to give rise to new major evolutionary lineages. In *Helianthus*, three of the four diploid hybrid species are rare endemics (Rieseberg, 1991a) and do not appear particularly likely to found major phylogenetic branches. However, the fourth and most ancient hybrid species, *H. bolanderi*, now consists of two distinct ecological races that have even been considered separate species by some authors (Rieseberg, 1991b; Oliveri & Jain, 1977). Nonetheless, the absence of allopolyploid species in this group makes it difficult to evaluate Grant's prediction.

The results from this study allow several conclusions. First, these results indicate that parental linkage groups are not retained intact in a diploid hybrid species (at least in the case of *H. anomalus*), but rather appear to be interspersed within individual chromosomes of the hybrid species. Second, these data raise the possibility that the parental genomic contributions to a diploid hybrid species may not necessarily be equivalent. These potential consequences of diploid hybrid speciation may increase the flexibility of a diploid hybrid, relative to an allopolyploid, in terms of optimizing its genome to a new ecological niche. Likewise, these same properties may lead to the production of several different diploid hybrid species from the same two parents, as appears to have occurred in *Helianthus*. Finally, these results demonstate the utility of the genomic mapping approach for the study of those genomic processes accompanying, facilitating, or resulting from different modes of speciation. Undoubtedly, the use of RAPD markers will greatly facilitate these types of studies.

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