Comparative Mapping and Rapid Karyotypic Evolution in the Genus Helianthus

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ABSTRACT

Comparative genetic linkage maps provide a powerful tool for the study of karyotypic evolution. We constructed a joint SSR/RAPD genetic linkage map of the *Helianthus petiolaris* genome and used it, along with an integrated SSR genetic linkage map derived from four independent *H. annuus* mapping populations, to examine the evolution of genome structure between these two annual sunflower species. The results of this work indicate the presence of 27 colinear segments resulting from a minimum of eight translocations and three inversions. These 11 rearrangements are more than previously suspected on the basis of either cytological or genetic map-based analyses. Taken together, these rearrangements required a minimum of 20 chromosomal breakages/fusions. On the basis of estimates of the time since divergence of these two species (750,000–1,000,000 years), this translates into an estimated rate of 5.5–7.3 chromosomal rearrangements per million years of evolution, the highest rate reported for any taxonomic group to date.

INTEREST in the evolution of genome structure can be found in both basic and applied research programs. Indeed, the fact that chromosomal rearrangements represent a barrier to the movement of genes between closely related taxa has made the study of karyotypic evolution a topic of great interest to evolutionary biologists and crop scientists alike (Stephens 1961; White 1978; Navarro and Barton 2003b). For an evolutionary biologist, the motivation is simple. Factors that restrict gene flow between populations allow for genetic differences to accrue, thus enabling the evolution of local adaptations and, ultimately, new species. Plant breeders have an equally clear reason for their interest in chromosomal rearrangements: any factor that limits the transfer of genes from one taxon to another limits the utility of wild germ plasm as a source of beneficial alleles for crop improvement. The conservation of gene order also facilitates the extrapolation of information from model species to less-studied crops (Gale and Devos 1998).

Until the late 1980s, studies of karyotypic evolution were largely based on cytological evidence; research on the phenomenon was therefore limited by the ability of researchers to visualize chromosomes. This all changed in 1988 when the first comparative linkage maps of eukaryotic genomes were published (Bonierbale *et al.* 1988; Chao *et al.* 1988; Tanksley *et al.* 1988), thereby initiating the field of plant comparative genetics. The increasing availability of molecular markers distributed throughout the genomes of a wide variety of taxa has enabled other researchers to use this approach in a variety of both plant (*e.g.*, Tanksley *et al.* 1992; Ahn and Tanksley 1993; Rieseberg *et al.* 1995; Devos and Gale 1997; Lagercrantz 1998; Livingstone *et al.* 1999; Wilson *et al.* 1999) and animal taxa (Serikawa *et al.* 1998; O'Brien *et al.* 1998, 1999; Gellin *et al.* 2000). The results of such studies have revealed that the extent of chromosome restructuring varies dramatically across different organismal groups, with considerable karyotypic change among closely related species in some lineages, but remarkable conservation of chromosome structure among divergent taxa in others. Overall, comparative map-based estimates of the rate of chromosomal evolution range from 0.2 to 2.5 structural rearrangements per million years of divergence (Lagercrantz 1998). Here we report on the rate of chromosomal evolution based on comparative genetic mapping in sunflowers.

The genus Helianthus is an economically and evolutionarily important taxon that contains not only one of the world's most important oilseed crops (the cultivated sunflower, *Helianthus annuus* L.), but also a number of wild species that have become increasingly important as models for the study of the genetics of adaptation and speciation (*e.g.*, Rieseberg *et al.* 1996; Lexer *et al.* 2003). In addition to their utility as models for studying evolutionary phenomena, wild species of Helianthus are thought to harbor a substantial amount of genetic diversity that may be appropriate for the improvement of the cultivated sunflower (Seiler and Rieseberg 1997). Indeed, wild Helianthus species are adapted to a wide range of habitats and possess substantial variability in a number of agronomically important traits (*e.g.*, Burke *et al.* 2002), suggesting that they might be a rich source of alleles for continued crop improvement.

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Unfortunately, genetic analyses within Helianthus have been hampered by the lack of sequence-specific, publicly available genetic markers. Maps of wild Helianthus species have been constructed with anonymous DNA markers such as randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (Rieseberg *et al.* 1993; Kim and Rieseberg 1999), which are typically difficult to compare across populations (but see Rieseberg *et al.* 1995). In contrast, cultivated sunflower maps have been largely based on codominant restriction fragment length polymorphism (RFLP) markers (*e.g.*, Gentzbittel *et al.* 1995; Berry *et al.* 1997; Jan *et al.* 1998), but the requisite probes are not publicly available. This situation improved dramatically with the recent publication of a large number of publicly available simple sequence repeat (SSR) markers (Tang *et al.* 2002; Yu *et al.* 2002). The availability of these tools makes the design and execution of comparative genetic studies within Helianthus much more feasible than ever before. In this study, we describe the construction of a joint SSR/RAPD map of the *H. petiolaris* genome and use it, along with an integrated SSR map derived from four independent *H. annuus* mapping populations, to investigate the evolution of genome structure within Helianthus.

MATERIALS AND METHODS

Study system: The genus Helianthus consists of 13 annual and 36 perennial species. Ploidy levels within the genus range from diploid (with a base chromosome number of $x = 17$) to hexaploid, with all of the annual species being diploid. As noted above, the focus here is on two of these annuals, *H. annuus* and *H. petiolaris*. Both *H. annuus* and *H. petiolaris* are widespread, polytypic species that exhibit numerous morphological and chromosomal differences (Heiser *et al.* 1969), occur in distinct clades based on both cpDNA and rDNA variation (Rieseberg 1991; Rieseberg *et al.* 1991), and have divergent habitat requirements. The former is typically restricted to heavy, clay soils, whereas the latter occurs mainly on dry, sandy soils. Previous analyses have suggested that these species differ by 7–10 chromosomal rearrangements, including two or three inversions and five to seven translocations (Chandler *et al.* 1986; Rieseberg *et al.* 1995). First-generation hybrids are semisterile, with pollen viabilities typically <10% and seed set <1% (Heiser 1947; Chandler *et al.* 1986). Aside from the obvious economic importance of *H. annuus* (see above), these two species have attracted considerable attention due to the fact that hybridization between them has resulted in the production of three diploid hybrid species (Rieseberg 1991). They also are sympatric throughout much of the western United States where hybrid swarms are common, making them an ideal system in which to study the effects of chromosomal rearrangements on gene flow (*e.g.*, Rieseberg *et al.* 1999).

Construction of the *H. petiolaris* map: The *H. petiolaris* map was generated from a population derived from an intraspecific *H. petiolaris* hybrid [Rieseberg 1104 × Seiler 1257; locality data in Beckstrom-Sternberg *et al.* (1991)] crossed against the inbred, male-sterile cmsHA89. The resulting mapping population consisted of 80 individuals and allowed for the segregation of *H. petiolaris* chromosomes to be tracked against a homozygous cultivar background [see Rieseberg *et al.* (1995) for additional details]. When it was originally constructed, this map consisted of 400 RAPD markers distributed across 17 linkage groups. In this study, we added 295 SSRs to the *H. petiolaris* map, thereby allowing us to compare the structure of *H. petiolaris* linkage groups against the integrated *H. annuus* SSR map described below. The SSR genotyping methodology followed previously established protocols (Burke *et al.* 2002), and markers were added to the map using MAPMAKER 3.0/EXP (Lander *et al.* 1987; Lincoln *et al.* 1992). Markers were initially divided into groups using the "group" command with LOD > 5, $\theta < 0.20$. The remaining markers were then assigned to groups by reducing the stringency to LOD > 3.0, $\theta < 0.25$. Map orders were explored using the "compare" and "try" commands and were confirmed using the "ripple" command. Recombination fractions were translated into centimorgan distances using Kosambi's (1944) mapping function.

Construction of the integrated *H. annuus* map: The composite *H. annuus* SSR map was constructed from four previously published maps of the species. The first of these was a map constructed from 94 recombinant inbred lines (RILs) derived from a cross between public confectionery and oilseed fertility restorer lines (RHA280 × RHA801). This map, which consists of 560 SSRs and 17 indels, contains 17 linkage groups and covers 1423 cM (Tang *et al.* 2002; Yu *et al.* 2003). The second map was constructed from 94 F_2 individuals derived from a cross between public oilseed fertility restorer and maintainer lines (HA370 × HA372). This map, which consists of 120 SSRs, 80 RFLPs, and two sequence-characterized amplified regions (SCARs), contains 17 linkage groups and spans 1275 cM (Gedil *et al.* 2001; Yu *et al.* 2003). The third map was constructed from 94 RILs derived from a cross between proprietary oilseed fertility restorer lines (PHA × PHB; developed by Pioneer Hi-Bred International, Woodland, CA). This map, which consists of 264 SSRs and one SCAR, coalesced into 20 linkage groups spanning 1200 cM (Yu *et al.* 2003). Three linkage groups (LG08, LG09, and LG16) were composed of two linkage subgroups apiece, thereby accounting for the discrepancy between the observed number of linkage groups and the actual number of chromosomes in *H. annuus* (20 *vs.* 17). The final map was constructed using 374 F_3 individuals derived from a cross between a public male-sterile breeding line and a single wild sunflower collected from Keith County, NE (cmsHA89 × Ann1238). This map, which consists of 202 SSRs, contains 17 linkage groups and spans 973 cM (Burke *et al.* 2002). Linkage groups in all four of these maps have been cross-referenced with each other on the basis of shared markers and are named following the standard nomenclature first introduced by Berry *et al.* (1997).

The four *H. annuus* maps were then integrated into a single, composite map as follows. A subset of markers from each of the preceding maps was first selected for inclusion in the integrated *H. annuus* map. Markers selected for this stage included those that fit one or more of the following criteria: (1) they were shared across two or more *H. annuus* maps, (2) they maximized coverage of the *H. annuus* genome, (3) they were informative (*i.e.*, shared) with respect to the *H. petiolaris* map, and/or (4) they were shared with maps of other wild Helianthus species (our unpublished data). This process resulted in the selection of 214, 102, 56, and 151 SSRs from each of the four preceding maps, respectively. In those rare instances where PCR primers produced multiple loci—only 13 such cases were included in this analysis, and only 1 of these involved an "informative" marker—orthology was inferred on the basis of linkage relationships with nearby, informative single-locus markers. The resulting data set consisted of 288 unique markers, of which 147 were shared across two or more of the individual *H. annuus* maps. The joint map was then

constructed using the software package JoinMap (version 3.0; Van Ooijen and Voorrips 2001). For map integration, linkage groups were first constructed for each map separately. These groups were then "joined" using the default settings and marker orders were reestimated on the basis of the joint data set. Recombination fractions were translated into centimorgan distances using Kosambi's (1944) mapping function.

Identification of chromosomal rearrangements: The *H. petiolaris* and *H. annuus* maps were aligned by hand, and chromosomal rearrangements were inferred on the basis of incongruities in the genomic locations and linear order of presumably orthologous SSRs.

RESULTS

Linkage maps: The *H. petiolaris* SSR/RAPD map spans 17 linkage groups and 1592 cM (supplementary Figure 1S, available at http://www.genetics.org/supplemental/). Of the 295 SSRs added to the *H. petiolaris* map, 91 were informative (*i.e.*, shared) with respect to the integrated *H. annuus* map. The remaining 204 SSRs were unique to the *H. petiolaris* map.

The integrated *H. annuus* linkage map is composed of 19 linkage groups spanning 828 cM (supplementary Figure 2S, available at http://www.genetics.org/supplemental/). In this case, the discrepancy between the actual number of chromosomes and the observed number of linkage groups is due to the fact that linkage groups 6 and 8 (LG06 and LG08) each consist of two unlinked subgroups. In both cases, the linkage subgroups are separated by considerable map distance on the individual *H. annuus* maps, and there was insufficient power in the joint data set to link them together. Their identities are, however, well established on the basis of shared markers. Thus, they are presented in the proper locations and orientations.

The overall map distance covered by the integrated map is substantially shorter than the individual *H. annuus* maps. This is partly due to the fact that two fairly sizable stretches of the *H. annuus* genome (corresponding to the middle of LG06 and LG08) are not represented in the total map distance—if the subgroups that comprise these groups had been successfully linked together in the integrated map, the total map length would have been increased by the distance separating the subgroups. Another factor contributing to the reduced length of the integrated map is that the cmsHA89 × Ann1238 map exhibits substantially shorter genetic distances over presumably equal physical distances when compared against the other *H. annuus* maps (~70% reduction in map distance across equivalent physical distances; Burke *et al.* 2002). This compression of map distance is likely due to reduced recombination in the cultivated × wild *H. annuus* cross employed by Burke *et al.* (2002) as compared to the narrower cultivar × cultivar crosses used in the production of the other maps. In view of the above, it is important to note that there is no evidence of variation in chromosome structure within or between cultivated and wild *H. annuus* (Heiser 1954; Chandler *et al.* 1986). Thus, while it may have resulted in a compression of the overall length of the integrated *H. annuus* map, the inclusion of the Burke *et al.* (2002) data allowed us to greatly increase the number of informative markers without influencing the inferred numbers/locations of chromosomal rearrangements.

To make a direct comparison of map lengths, we analyzed the distance separating the outermost shared markers in colinear segments of the Tang *et al.* (2002) and *H. petiolaris* maps. Our rationale for using the Tang *et al.* map as our *H. annuus* reference was that it does not exhibit the compression of map length characteristic of the wider Burke *et al.* (2002) cross, it has the most complete marker coverage of the three cultivar × cultivar maps, and it coalesced into the expected 17 linkage groups. Overall, we were able to compare map lengths in 14 of the 27 regions of colinearity [see below for details on the identification of colinear segments; also note that "rogue" markers (also below) were excluded from this analysis]. In total, the 14 intervals spanned 499 cM in *H. annuus* and 448.5 cM in *H. petiolaris* (Table 1). One of these intervals was identical in length between the two maps, whereas 8 were longer in *H. petiolaris* and 5 were longer in *H. annuus*. Neither of the above results was statistically significant (pairwise *t*-test, $P = 0.63$, d.f. 13; sign test, $P > 0.10$).

Linkage group comparisons: The comparison of linkage group composition and marker ordering revealed the presence of seven colinear linkage groups (LG01, LG03, LG04, LG07, LG09, LG10, and LG11) with the remainder of the *H. annuus* linkage groups being rearranged to a greater or lesser extent in *H. petiolaris*. Representative comparisons for colinear and rearranged linkage groups are presented in Figure 1, and an illustration of linkage group relationships across the entire genome is presented in Figure 2.

Inspection of Figure 2 reveals the presence of 27 colinear segments resulting from eight translocations and three inversions that, taken together, required a minimum of 20 chromosomal breakages/fusions. More specifically, in *H. petiolaris*, LG06 and LG15 are fused; LG08 has been split into two segments, one of which is associated with LG02, whereas the other is associated with a portion of LG16; the balance of LG16 is split into two separate linkage groups; LG12 is split into two segments, one of which remains independent, while the other is associated with a portion of LG14; the remainder of LG14 is associated with LG05; and finally, LG17 is split into two separate segments, one of which remains independent, whereas the other is associated with LG13. Note that, although the density of informative SSRs was, in some cases, too low to corroborate the previously identified inversions, the linear ordering of RAPD markers inferred by Rieseberg *et al.* (1995) in these regions remained consistent in the presence of the SSR data, thereby supporting the presence of the three inversions

TABLE 1

Comparison of map lengths (centimorgans) of colinear segments in the *H. annuus* and *H. petiolaris* genomes

Linkage group	Outermost markers	*H. annuus*	*H. petiolaris*	Difference
LG01	ORS0610-ORS0959	34.5	11.3	23.2
LG02	ORS0229-ORS0423	39.3	46.1	−6.8
LG03	ORS0545-ORS0822A	69	105.5	−36.5
LG05	ORS0852-ORS0547	24.9	61.8	−36.9
LG07	ORS0966-ORS0928	33.9	1.3	32.6
LG08	ORS0166-ORS1043	0	3.9	−3.9
LG09	ORS1034-ORS1001	86.6	49.2	37.4
LG10	ORS0878-ORS1048	61.7	61.7	0
LG11	ORS1147-1-ORS0768	99.9	45.4	54.5
LG12	ORS1040A-ORS0946	1.8	3.8	−2
LG14	ORS1086-ORS0832	14.3	18.9	−4.6
LG15	ORS0007-ORS0008	0	5.2	−5.2
LG16	ORS0768-ORS1017	8.9	33.1	−24.2
LG16	ORS1198-ORS0378	24.2	1.3	22.9
Total		499	448.5	50.5

identified by those authors. The inversions on LG12 and LG13 appear to be terminal, such that they can each be explained by a single breakage and a single fusion. The inversion on LG16 required two breaks and two fusions.

The only other discrepancies in terms of marker order between *H. annuus* and *H. petiolaris* consisted of individual markers that mapped to the wrong position on the right linkage group. In some cases, these were extremely localized ordering differences that spanned one or, at most, a few centimorgans, whereas others involved grossly misplaced rogue markers. Because it is very difficult to discern the precise order of tightly linked clusters of markers and because the integration of four disparate data sets might introduce inconsistencies into the subsequent analyses, single-marker ordering differences such as these were ascribed to mapping error rather than to structural rearrangements. It is also possible that some of the rogue markers represented loci that were paralagous to those mapped in *H. petiolaris*.

DISCUSSION

Map lengths: As described above, the integrated *H. annuus* map was substantially shorter than the *H. petiolaris* map. However, a comparison of the distances separating markers shared between the Tang *et al.* (2002) and *H. petiolaris* maps revealed that there is no detectable difference in the lengths of colinear intervals across the genomes of these two species. When combined with the fact that *H. annuus* and *H. petiolaris* are believed to have virtually identical genome sizes (Sims and Price 1985), this result indicates that the recombination rate per unit of physical distance is similar in the two species. However, in view of the heterogeneity in length differences among genomic regions (Table 1), interval- or linkage group-specific differences in recombination rates between the two species remain a possibility.

Extent of chromosomal repatterning: In terms of the number of chromosomal rearrangements differentiating *H. annuus* and *H. petiolaris*, the 11 rearrangements (eight translocations and three inversions) reported here are more than previously suspected on the basis of either cytological or genetic map-based analyses. Indeed, we detected one more inversion and three more translocations than inferred by Chandler *et al.* (1986) and identified a translocation (LG08/LG16) that had been missed in earlier work by Rieseberg *et al.* (1995). Of course, these 11 rearrangements are most likely a somewhat conservative estimate of the true extent of chromosomal divergence between *H. annuus* and *H. petiolaris*. Because the ability to detect chromosomal rearrangements via comparative linkage mapping is limited by marker density, large-scale rearrangements are readily detected, whereas those involving small chromosomal segments are likely to go undetected. In addition, although we attributed single-marker incongruities to either mapping error or the amplification of paralogous loci, it is possible that some fraction of these resulted from the transposition of small chromosomal fragments from one region to another. In either case, the actual level of colinearity between *H. annuus* and *H. petiolaris* is probably somewhat lower than the 27 colinear segments averaging 59 cM each that are reported here.

Rates of chromosomal evolution: Rates of chromosomal evolution are known to be heterogeneous across taxa, with previously published comparative map-based estimates ranging from 0.2 to 2.5 structural rearrangements per million years of divergence (Table 2; Lagercrantz 1998). When viewed in this context, it is clear that *H. annuus* and *H. petiolaris* have experienced extraordinarily rapid chromosomal evolution in the time

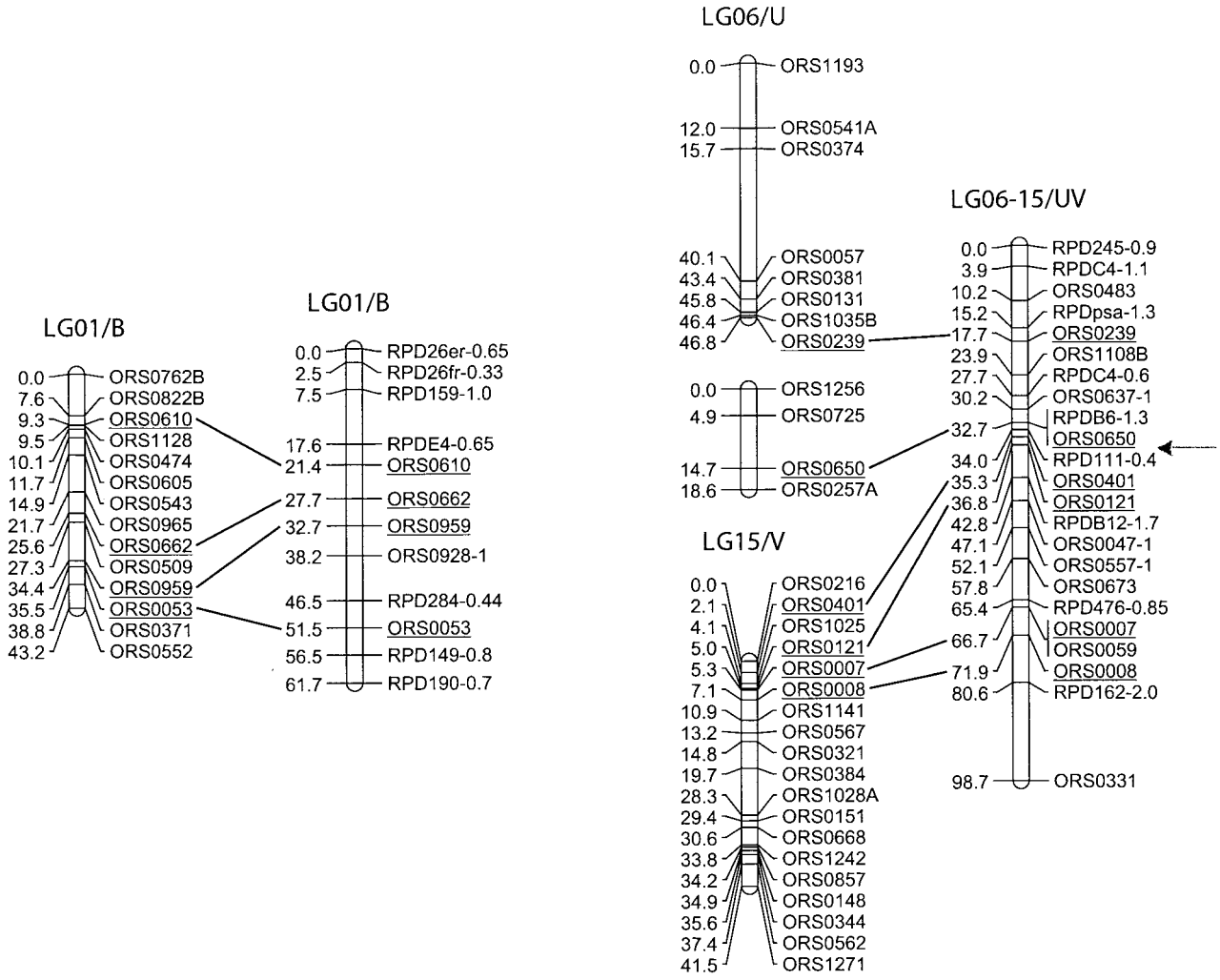

FIGURE 1.—Representative linkage group comparisons between the sunflower species *H. annuus* and *H. petiolaris*. The comparison on the left depicts linkage group one (LG01), which is colinear in *H. annuus* (left) and *H. petiolaris* (right). The comparison on the right depicts the fusion of LG06 and LG15 from *H. annuus* (left) in *H. petiolaris* (right). All marker names beginning with ORS refer to simple sequence repeats, whereas those beginning with RPD refer to RAPDs. Numbers to the left of each linkage group refer to genetic distance (centimorgans). For each comparison, informative loci are underlined and connected by lines. Arrow on the far right indicates the location of the inferred chromosomal breakage/fusion that distinguishes *H. annuus* from *H. petiolaris*. Letters following numerical linkage group designations refer to the naming system used by RIESEBERG *et al.* (1995) and are included here for continuity.

since they diverged. Indeed, on the basis of levels of chloroplast sequence divergence, the estimated time since these species shared a common ancestor is 750,000–1,000,000 years (RIESEBERG *et al.* 1991), meaning that the 11 structural rearrangements documented here accrued at a rate of 5.5–7.3 rearrangements per million years of evolution.

Why has chromosomal evolution occurred so rapidly in sunflowers? One possibility is that the apparently low rates in certain other taxonomic groups may, at least in part, be an artifact of generation time. *H. annuus* and *H. petiolaris* are both annuals, meaning that their 750,000- to 1,000,000-year history corresponds to a similar number of generations. The much lower rates of chromosomal evolution characteristic of some groups (*e.g.*, Homo-Mus) would be considerably higher if they were calculated on a per generation basis. This estimate does, however, remain two to three times higher than that of even the most rapidly evolving annual plant species, *Brassica rapa* and *B. oleracea* (Table 2).

Another possibility is that certain aspects of their biology predispose these species to high rates of chromosomal evolution. For example, although the annual species of Helianthus (including *H. annuus* and *H. petiolaris*) exhibit regular bivalent formation at meiosis, HEISER and SMITH (1955) suggested that the base chromosome number within the genus ($x = 17$) might be of ancient polyploid origin, with the ancestral number of chromosomes within the sunflower family (*i.e.*, the Asteraceae) being $x = 9$. JACKSON and MURRAY (1983) later provided evidence supporting HEISER and SMITH's (1955) hypothesis of paleopolyploidy. It has been argued else-

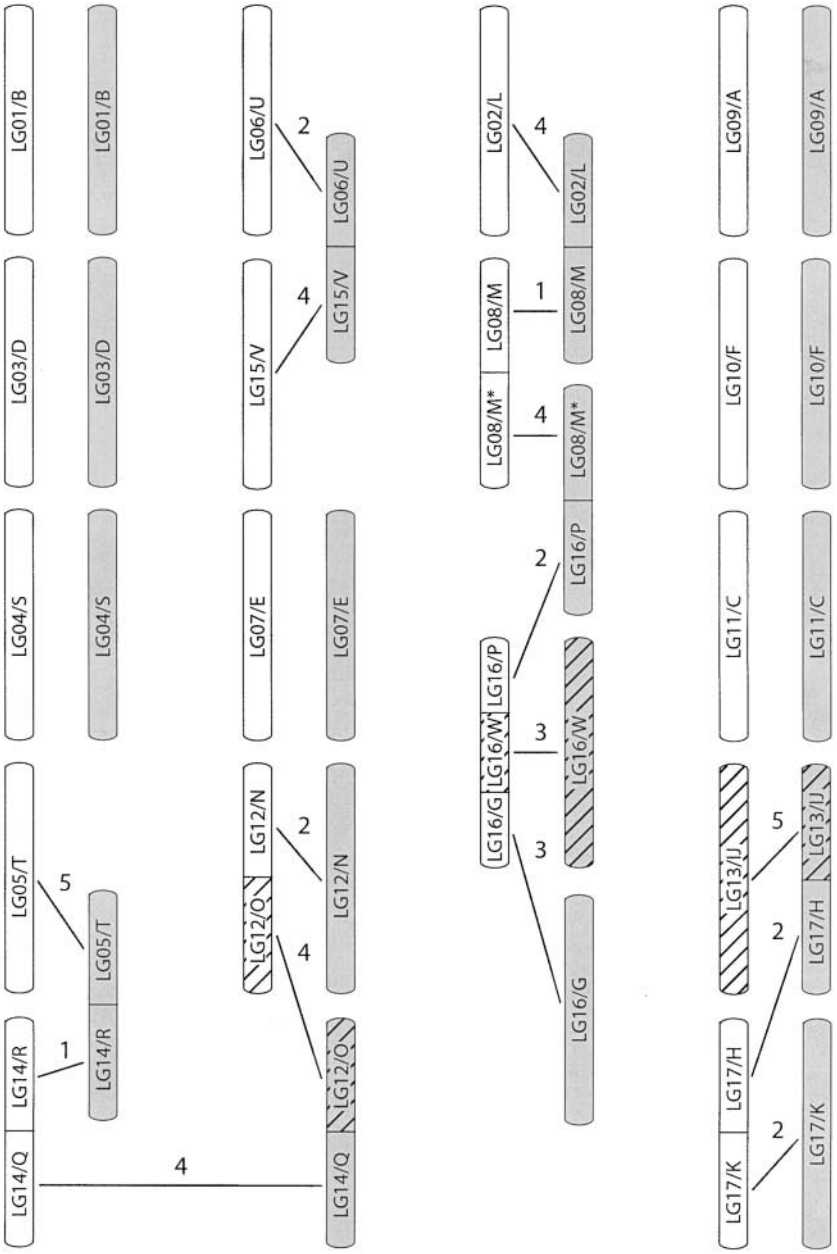

FIGURE 2.—Diagram of inferred structural relationships between chromosomes of *H. annuus* and *H. petiolaris*. *H. annuus* linkage groups are depicted in white, whereas *H. petiolaris* linkage groups are shaded. The homologous segments of all rearranged linkage groups are connected with lines. Numbers above these lines indicate the number of markers supporting each of the rearranged segments. Segments containing inversions are denoted with hatched lines.

where that polyploidy may result in aberrant meiotic pairing and translocations among homeologous chromosomes (LAGERCRANTZ 1998). Also, chromosomal rearrangements may be established more easily in polyploid taxa than in diploids because heterozygosity for rearrangements is less likely to cause sterility in redundant genomes (RIESEBERG 2001). However, it must be kept in mind that the polyploidization event underlying Helianthus predates the origin of the genus and likely occurred at least 6 million years ago (SCHILLING *et al.* 1994). Although little is known about the time required for diploidization, it is likely that the process was well on its way prior to the divergence of *H. annuus* and *H. petiolaris*, and thus polyploidy may not have played a major role in their karyotypic divergence.

Assuming that sunflowers exhibit a "typical" rate of chromosomal mutation, then the rate of karyotypic evolution will depend on (1) the fitness effects of structural rearrangements and (2) genetic drift (LANDE 1979). The classic view of chromosomal rearrangements is that, although they have no effect on fitness when homozygous, they are typically deleterious when heterozygous (MULLER 1954, 1956; WHITE 1973). Assuming this to be true, the fixation of such rearrangements requires that genetic drift overwhelms selection against heterozygotes. Although both *H. annuus* and *H. petiolaris* are widespread species, with ranges covering much of North America, their populations fluctuate dramatically in size (STEBBINS and DALY 1961; CARNEY *et al.* 2000) and are prone to high levels of population turnover (HARRISON *et al.* 2000). Thus, the population dynamics of these species are right for the fixation of chromosomal rearrangements

TABLE 2

Estimated divergence times, numbers of chromosomal rearrangements, and rates of chromosomal evolution for various taxa

Taxa	Divergence time (millions of years)	No. of rearrangements	Rate[a]
H. annuus-H. petiolaris	0.75–1	11	5.5–7.3
B. rapa-B. oleracea	1	5	2.5
A. thaliana-B. nigra	35	90	1.3
Homo-Mus	114	144	0.6
Gossypium spp.	10	9	0.5
Sus-Mus	114	77	0.3
Sorghum-Zea	24	15	0.3
B. nigra-B. oleracea	20	12	0.3
Oryza-Zea	66	35	0.3
B. nigra-B. rapa	20	10	0.3
Lycopersicon-Solanum	10	5	0.3
Homo-Sus	93	35	0.2
Lycopersicon-Capsicum	40	14	0.2

Helianthus data are from this study. All other divergence times and numbers of rearrangements are from Lagercrantz (1998).

[a] Rate was calculated as (number of rearrangements)/(2 × divergence time) because rearrangements can accrue on the branch leading to either of the two taxa.

via genetic drift in small, local populations. Once established, underdominant rearrangements can easily spread via repeated bouts of localized extinction and recolonization (Lande 1979).

Another possibility is that the high rate of chromosomal evolution documented here is a byproduct of the differential survival of incipient neospecies or so-called isolate selection (Stanley 1979). Eldredge (1989) proposed that isolate selection favors ecologically divergent taxa because they face reduced competition with their progenitor. Levin (2000) later extended this idea to the reproductive realm; neospecies with stronger barriers to hybridization are less likely to go extinct due to "reproductive interference" than are taxa that are reproductively compatible with their progenitor. Reproductive isolate selection may have played a role in the formation of one or both of these species. Because the habitats of the annual sunflowers are often juxtaposed, it is possible that only a very strongly isolated neospecies would be able to withstand the challenge of parapatry with the progenitor species. Weakly isolated populations of "proto-*annuus*" or "proto-*petiolaris*" would merge back into the ancestral species, leaving only the most divergent form(s) to give rise to what we now recognize as *H. annuus* and *H. petiolaris*.

A final possibility is that chromosomal rearrangements may have little initial impact on fitness, making their fixation much more likely than might otherwise have been predicted. There are two possibilities here. First, individual rearrangements may be only weakly underdominant, thereby aiding in their establishment, but strongly underdominant in combination, thereby producing reproductive isolation. For example, Baker and Bickham (1986) showed that monobrachial (*i.e.*, single arm) centric fusions are readily fixed in natural populations because trivalents can form at meiosis and segregate normally. However, a strong sterility barrier can develop between populations fixed for different fusions with monobrachial homology (*i.e.*, one arm is homologous, the other is not). Unfortunately, without knowing the details of the ancestral karyotype, it is difficult to tell if something similar has happened in Helianthus. Alternatively, chromosomal rearrangements may have little or no initial impact on fitness because of mechanisms that alleviate or prevent abnormal segregation at meiosis (such as partial or complete suppression of recombination; Coyne *et al.* 1993) or because of genomic redundancy (Rieseberg and Livingstone 2003). However, as rearrangements begin to accrue genic incompatibilities (*e.g.*, Noor *et al.* 2001) or diverge at duplicate genes (Werth and Windham 1991; Lynch and Force 2000), their effects might become more severe, ultimately producing the observed decrease in hybrid fitness (Rieseberg 2001; Rieseberg and Livingstone 2003). In Helianthus, interspecific hybrids typically exhibit multivalent configurations and abnormal segregation at meiosis (Chandler *et al.* 1986), suggesting that genomic redundancy provides the most plausible explanation for any possible reduction in the initial fitness effects of rearrangements.

The distribution of rearrangements across the genome: Whatever the cause, the high rate of chromosomal evolution between *H. annuus* and *H. petiolaris* suggests that few, if any, regions of their genomes are protected from rearrangement. Indeed, if large portions of the genome were protected from rearrangement, perhaps due to reg-

ulatory or functional interactions among linked sets of loci, then we might expect the rate of chromosomal evolution to be relatively low. On the other hand, in spite of the accumulation of a substantial number of rearrangements, we observed seven completely colinear chromosomes. The question, then, is whether or not we would expect to see this many colinear linkage groups if the rearrangements were distributed at random across the genome. Because we do not know the nature of the ancestral karyotype, we cannot unequivocally assign chromosomal breakages and fusions to individual linkage groups within *H. annuus* or *H. petiolaris*. Thus, we are limited to asking questions about the colinear linkage groups. For example, assuming that the 20 inferred chromosomal breakages and fusions were distributed randomly across the genome, we can ask what is the likelihood that we would see seven or more completely colinear linkage groups? In this case, computer simulations fail to reject the hypothesis of a random distribution of breakages and fusions ($P = 0.16$, data not shown), suggesting that there is little (if any) constraint on where in the genome rearrangements can occur.

Implications and opportunities: Despite the high rate of chromosomal evolution between these species, their relatively young age results in the retention of large stretches of colinearity, a fact that suggests that wild germ plasm can be exploited for the continued improvement of cultivated sunflower. A more complete understanding of genome evolution within Helianthus, however, awaits finer-scale analyses of not only *H. annuus* and *H. petiolaris*, but also other Helianthus species. Fortunately, the tools are now in place to extend these sorts of analyses across the genus to determine if the observed high rate of karyotypic evolution is typical of the genus as a whole or if it is restricted to particular species or to groups of species. Issues remaining to be addressed include whether or not rates of chromosomal evolution within the genus vary as a function of life history (*e.g.*, annual *vs.* perennial habit), ploidy level, and geographic distribution.

This system also is ideal for testing a new model of chromosomal speciation, which emphasizes the reduction of recombination in chromosomes heterozygous for the rearrangements (Noor *et al.* 2001; Rieseberg 2001; Navarro and Barton 2003a). The reduction in recombination may be due to actual suppression of recombination in rearranged regions (Coyne *et al.* 1993) or to selection again recombinant gametes. Either mechanism leads to reduced rates of introgression in rearranged chromosomes (Rieseberg *et al.* 1999) and the differential accumulation of species differences (Noor *et al.* 2001; Navarro and Barton 2003b). Studies are currently underway to determine whether rates of protein evolution and divergence in gene expression profiles are associated with the occurrence of a gene in colinear *vs.* rearranged portions of the genome.

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