Karyotypic Evolution of the Common and Silverleaf Sunflower Genomes

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Abstract

Silverleaf sunflower (*Helianthus argophyllus* Torrey and Gray) has been an important source of favorable alleles for broadening genetic diversity and enhancing agriculturally important traits in common sunflower (*H. annuus* L.), and, as the closest living relative of *H. annuus*, provides an excellent model for understanding how apparently maladaptive chromosomal rearrangements became established in this genus. The genomes of *H. annuus* and *H. argophyllus* were comparatively mapped to identify syntenic and rearranged chromosomes and develop genomic blueprints for predicting the impact of chromosomal rearrangements on interspecific gene flow. Syntenic chromosomal segments were identified and aligned using 131 orthologous DNA marker loci distributed throughout the *H. annuus* genome (299 DNA marker loci were mapped in *H. argophyllus*). We identified 28 colinear chromosomal segments, 10 colinear chromosomes, and seven chromosomal rearrangements (five non-reciprocal translocations and two inversions). Four *H. argophyllus* chromosomes carrying non-reciprocal translocations apparently arose from the duplication of two chromosomes, and three *H. argophyllus* chromosomes apparently arose from end-to-end or end-to-opposite-end fusions of chromosomes or chromosome segments. Chromosome duplication may reduce the initial fitness costs of chromosomal rearrangements, thereby facilitating their establishment. Despite dramatic differences in chromosome architecture, a significant fraction of the *H. argophyllus* genome appears to be accessible for introgression into *H. annuus*.

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CROSS-TAXA SYNTENY analyses in the Poaceae, Solanaceae, Cruciferae, Fabaceae, and other plant families have uncovered conserved gene orders among taxonomically divergent plant species. Such analyses have facilitated the application of model species genomic resources for identifying and cloning loci underlying biologically and agriculturally important phenotypes across taxonomic boundaries (Bonierbale et al., 1988; Tanksley et al., 1988; Chao et al., 1989; Rieseberg et al., 1995a; Lagercrantz, 1998; Wilson et al., 1999; Devos and Gale, 2000; Paterson et al., 2000; Doganlar et al., 2002; Koch and Kiefer, 2005; Yogeeswaran et al., 2005). The development of high-throughput DNA marker genotyping technologies, coupled with comparative genetic mapping, have been powerful tools for identifying chromosomal rearrangements among species, understanding karyotypic evolution, predicting the impact of chromosomal rearrangements (translocations and inversions) on interspecific gene flow, and developing strategies for introgressing wild species alleles into modern cultivars through marker-assisted selection (MAS) (Rieseberg et al., 1995a,1995b; Noor et al., 2001; Zamir, 2001; Burke et al., 2004; Koch and Kiefer, 2005; Yogeeswaran et al., 2005; Lai et al., 2005b).

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Abbreviations: INDEL, insertion-deletion; IL, introgression line; MAS, marker-assisted selection; MYA, million years ago; NMS, nuclear male-sterile; QTL, quantitative trait locus; *R*, resistance; SNP, single nucleotide polymorphism; SSCP, single strand conformational polymorphism; SSR, simple sequence repeat.

The development of several hundred DNA sequence-based markers for common sunflower (*Helianthus annuus* L.; 2n = 2x = 34) has facilitated comparative mapping of the genomes of several wild species, and genome-wide analyses of the role of karyotypic evolution in sunflower speciation (Tang et al., 2002, 2003; Yu et al., 2002, 2003; Burke et al., 2004; Lai et al., 2005a; Heesacker et al., 2008; Radwan et al., 2008). The genomes of *H. annuus*, *H. petiolaris* Nutt. (2n = 2x = 34), and three *H. annuus* × *H. petiolaris* homoploid (2n = 2x = 34) hybrid species (*H. anomalus* Blake, *H. deserticola* Heiser, and *H. paradoxus* Heiser) have been comparatively mapped (Rieseberg et al., 1995a; Burke et al., 2004; Lai et al., 2005b) using a common collection of simple sequence repeat (SSR) markers (Tang et al., 2002, 2003; Yu et al., 2002, 2003). Despite the relatively recent divergence of these species (Schwarzbach and Rieseberg, 2002; Welch and Rieseberg, 2002; Gross et al., 2003; Strasburg and Rieseberg, unpublished data, 2009), their genomes are distinguished by a phenomenal number of chromosomal rearrangements: 9 to 11 of the 17 linkage groups were non-syntenic in pairwise comparisons, and only four of the 17 linkage groups were colinear across species (Burke et al., 2004; Lai et al., 2005b). Burke et al. (2004) estimated the rate of karyotypic evolution in *H. annuus–H. petiolaris* at 5.5 to 7.3 chromosomal rearrangements/MYA, higher than previously reported for other eukaryotic genera. Even though *H. annuus*, *H. petiolaris*, and their homopoloid hybrid species are distinguished by multiple chromosome rearrangements and reduced interspecific hybrid fertility (Rieseberg et al., 1995a; Burke et al., 2004; Lai et al., 2005b), these species are interfertile and supply a wealth of genetic diversity for sunflower breeding (Rogers et al., 1982; Chandler et al., 1986).

The high rates of karyotypic evolution in *Helianthus* and in many other plant species represent one of the oldest unsolved mysteries in genome evolution: Why are chromosomal rearrangements that reduce fitness when heterozygous (i.e., underdominant rearrangements) much more frequent in plants than in animals (Dobzhansky, 1933; Rieseberg, 2001; Coyne and Orr, 2004)? Population genetic theory indicates that such rearrangements should be extremely rare regardless of taxon (Hedrick, 1981; Walsh, 1982; Lande, 1985), yet underdominant rearrangements appear to be common in many plant groups, including *Helianthus*, in which quantitative trait loci (QTL) for sterility have been found to map to chromosomal breakpoints (Quillet et al., 1995; Lai et al., 2005b). Because *Helianthus* species are self-incompatible outcrossers with huge effective population sizes (Strasburg and Rieseberg, 2008), traditional explanations involving very small populations and selfing are not tenable (Hedrick, 1981; Walsh, 1982; Lande, 1985; Coyne and Orr, 2004). As the closest relative of common sunflower (Schilling and Heiser, 1981; Rieseberg et al., 1991), comparative genome analysis of silverleaf sunflower (*H. argophyllus* Torrey and Gray; 2n = 2x = 34) offers an excellent opportunity for addressing this mystery.

Silverleaf sunflower, which diverged from *H. annuus* 0.74 to 1.67 MYA (Strasburg and Rieseberg, unpublished data, 2009), has been widely used as a donor of novel disease-resistance alleles in sunflower breeding programs, despite reduced fertility and meiotic abnormalities in hybrid offspring (Heiser, 1951; Miller and Gulya, 1988; 1991; Besnard et al., 1997; Miller et al., 2002; Radwan et al., 2003; Slabaugh et al., 2003; Dussle et al., 2004). Most commonly, *H. argophyllus* alleles have been introgressed into *H. annuus* through phenotypic selection and backcrossing without identifying or tracking the genomic locations of introgressed segments. Genetic mapping in interspecific (*H. annuus* × *H. argophyllus*) hybrid populations and graphical genotyping of wild introgression lines (ILs) using SSR, insertion-deletion (INDEL), and single-strand conformational polymorphism (SSCP) markers have identified the genomic locations of two *H. argophyllus* introgressions harboring downy mildew [*Plasmopara halstedii* (Farl.) Berl. and de Toni] resistance (*R*) genes (Slabaugh et al., 2003; Dussle et al., 2004). RHA340, an IL developed by phenotypic selection for resistance to downy mildew races 2, 3, and 4 (Miller and Gulya, 1988), carries an *H. argophyllus* introgression on linkage group 13 harboring a large cluster of downy mildew *R*-genes (Bouzidi et al., 2002; Radwan et al., 2003, 2004, 2008; Slabaugh et al., 2003). ARG1575-2, an IL developed by phenotypic selection for resistance to downy mildew races 300, 700, 730, and 770 in an interspecific (*H. annuus* × *H. argophyllus*) population, carries a *H. argophyllus* introgression on linkage group 1 harboring Pl_{ARG}, another downy mildew *R*-gene (Dussle et al., 2004). Other than the downy mildew resistance loci found on linkage groups 1 and 13, the genomic locations of other agriculturally important loci introgressed from *H. argophyllus* are not known.

From meiotic abnormalities identified by cytological analyses of pollen mother cells, the chromosomes of *H. annuus* and *H. argophyllus* have been predicted to differ by two reciprocal translocations (Chandler et al., 1986; Quillet et al., 1995). This prediction has not been substantiated by comparative mapping, as discussed below. Chromosomal rearrangements complicate breeding in interspecific populations by reducing hybrid fertility and disrupting meiotic pairing and recombination. Moreover, recombination is often suppressed in syntenic chromosomes or chromosomal segments among interspecific hybrid offspring. These problems are typically alleviated by backcrossing partially fertile hybrid individuals to an elite recurrent parent and selecting among backcross and advanced backcross progeny (Tanksley and Nelson, 1996; Zamir, 2001).

Backcross breeding strategies have been widely applied in *H. annuus* × *H. argophyllus* populations without understanding the consequences of chromosomal rearrangements on the transmission of alleles across the species barrier. Moreover, the spectrum of chromosomal segments recovered in *H. annuus* × *H. argophyllus* breeding programs is not known. The genomes of *H. argophyllus* and *H. annuus* were comparatively mapped in the present

study to identify chromosomal rearrangements, gain additional insights into karyotypic evolution in sunflower, and develop 'genomic blueprints' to facilitate MAS introgression of *H. argophyllus* alleles into *H. annuus*.

Materials and Methods

Mapping Population Development

We developed an interspecific hybrid (F_1) testcross mapping population by crossing a single male-sterile individual from a nuclear male-sterile (NMS) *H. annuus* inbred line, NMS801 (Miller, 1992), with a single randomly selected male-fertile individual from an outbred *H. argophyllus* population (ARG1805-2 = PI 494571). F_1 seeds were harvested and germinated at 22°C on moistened blotter paper. Leaf samples were collected and lyophilized from 94 four-week-old greenhouse grown F_1 plants. DNA was isolated from lyophilized leaf samples using a modified CTAB method (Murray and Thompson, 1980) and DNA concentrations were quantified on a BioTek Synergy HT Microplate Spectrophotometer (BioTek Instruments, Winooski, VT).

DNA Marker Genotyping

Several hundred previously mapped and unmapped SSR, INDEL, and SSCP markers were screened for polymorphisms between NMS801 and a bulk of 20 NMS801 × ARG1805-2 F_1 progeny (Tang et al., 2002; Yu et al., 2003; Heesacker et al., 2008; Radwan et al., 2008; http://www.sunflower.uga.edu/cmap/ verified 7 Aug. 2009). SSR, INDEL, and SSCP alleles were amplified using touchdown PCR (Don et al., 1991). Forward primers were end-labeled with FAM, HEX, or TET fluorophores, whereas reverse primers were unlabeled for SSR and INDEL markers. SSR markers were screened for length polymorphisms on an ABI Prism 377 Automated DNA Sequencer and genotyped in the *H. argophyllus* mapping population (n = 94) on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) using Filter Set D and the ROX 500 internal-lane standard. SSR markers were multiplex genotyped in the mapping population by pooling amplicons produced by 6 to 16 SSR markers. SSR genotypes (allele lengths) were recorded and called using GeneScan 2.1 and Genotyper 2.0 (Applied Biosystems). INDEL markers were screened for polymorphisms and genotyped on agarose or on an ABI 3730XL DNA Analyzer as described for SSR markers. SSCP markers were screened for polymorphisms and genotyped in the mapping population using mutation detection enhancement (MDE) polyacrylamide electrophoresis (Martins-Lopes et al., 2001; Bertin et al., 2005; Radwan et al., 2008) and were multiplex genotyped in the mapping population by pooling amplicons produced by two to four SSCP markers. DNA fragments were electrophoretically separated for 16 h at a constant power of 8 W at room temperature and silver-stained (Bassam et al., 1991).

Genetic Mapping and Macrosynteny Analyses

Statistical and genetic mapping analyses were performed on interspecific F_1 testcross progeny DNA marker genotypes using expected segregation ratios and mapping functions developed for backcross populations (Rieseberg et al., 1995b). By genotyping interspecific F_1 testcross individuals, intraspecific (*H. argophyllus*) mapping information was produced by tracking segregation and recombination at heterozygous loci in the outbred male (*H. argophyllus*) parent. Heterozygous loci ($A^{ARG}a^{ARG}$) in the male parent (ARG1805-2, a single *H. argophyllus* individual) were expected to segregate 1 $A^{ANN}A^{ARG}$:1 $A^{ANN}a^{ARG}$ among F_1 progeny, where A^{ANN} was the allele transmitted by the *H. annuus* parent ($A^{ANN}A^{ANN}$) and A^{ARG} and a^{ARG} were alleles transmitted by the heterozygous *H. argophyllus* parent ($A^{ARG}a^{ARG}$). Heterozygous loci in the *H. argophyllus* parent ($A^{ARG}a^{ARG}$) were genotyped and mapped in the F_1 testcross population when the *H. annuus* parent was homozygous for one or neither of the alleles transmitted by the *H. argophyllus* parent. DNA marker loci were grouped and ordered using backcross mapping functions and supplied intraspecific mapping information for identifying syntenic chromosomal segments and chromosomal rearrangements between *H. annuus* and *H. argophyllus* (Rieseberg et al., 1995b). DNA markers were screened for segregation distortion using χ^2–statistics (Bailey, 1961). DNA marker loci were grouped and ordered, and genetic distances (cM) were estimated using MAPMAKER (Lander et al., 1987). Groups were found using a likelihood of odds (LOD) threshold of 3.0 and minimum recombination frequency threshold of 0.40. Orders were found and compared using the ORDER and RIPPLE commands in MAPMAKER under the assumptions of 0% or 1% genotyping errors (Lander et al., 1987; Lincoln and Lander, 1992). Genetic distances were estimated using the Kosambi mapping function (Kosambi, 1944). The LOD threshold for grouping loci was relaxed to 2.0 in a second analysis to identify putative subgroups of linkage groups found using LOD = 3.0.

Sunflower Linkage Group Naming Conventions

H. annuus and *H. argophyllus* linkage groups were aligned using orthologous DNA marker loci mapped in both species. *H. annuus* linkage groups were numbered and oriented using standard public linkage group nomenclature (Tang et al., 2002; Yu et al., 2003; http://www.sunflower.uga.edu/cmap/). ANN was added as a prefix to *H. annuus* linkage group numbers (1 = ANN1, 2 = ANN2... 17 = ANN17) to facilitate cross-species comparisons. Several rules were applied when naming *H. argophyllus* linkage groups and identifying colinear and rearranged chromosomal segments. First, *H. argophyllus* linkage groups, identified by the prefix ARG, were numbered and oriented using the standard linkage group nomenclature for *H. annuus* (Tang et al., 2002; Yu et al., 2003; http://www.sunflower.uga.edu/cmap/). Second, colinear linkage groups were assigned

identical numbers, e.g., ARG2 is colinear with ANN2. Third, colinear linkage subgroups were assigned identical numbers with capital letters (A and B), e.g., two *H. argophyllus* linkage groups were colinear with upper and lower segments of ANN1 and were identified as linkage subgroups ARG1A and 1B. Fourth, two *H. argophyllus* linkage groups sharing overlapping subsets of independent or duplicated DNA marker loci from a single *H. annuus* linkage group were identified using numerical suffixes, e.g., two *H. argophyllus* linkage groups shared overlapping subsets of independent DNA marker loci from ANN14 and were identified as ARG14-1 and 14-2. Fifth, *H. argophyllus* linkage groups produced by the fusion of two *H. annuus* linkage groups were identified by using linkage group numbers from the fused groups, e.g., ARG6/15 was produced by the fusion of ANN6 and ANN15. Sixth, inverted locus orders spanning short segments (<4 cM) were identified as 'local' locus ordering differences caused by genotyping or statistical errors and were not identified as inversions. Seventh, inversions were only proposed when supported by two or more shared orthologous loci spanning segments longer than 4.0 to 6.0 cM, and were identified by the suffix INV. Eighth, loci mapping to grossly different positions within a linkage group were identified as 'rogue loci' and could have either been accurately mapped paralogous loci or inaccurately mapped orthologous loci. Regardless, rogue loci were not identified as orthologous for macrosynteny analyses. Ninth, duplicated loci were identified by adding letters as locus name suffixes (A, B, or C).

Linkage groups previously identified by comparative mapping in *H. annuus*, *H. petiolaris*, *H. anomalus*, *H. deserticola*, and *H. paradoxus* (Burke et al., 2004; Lai et al., 2005b) were aligned with *H. argophyllus* linkage groups identified in the present study. Using macrosynteny among these linkage groups for the identification of shared and unique chromosomal rearrangements among species and estimates of phylogenetic divergence times (Rieseberg et al., 1991), rates of karyotypic evolution (*K*) were estimated for *H. annuus–H. argophyllus* and *H. petiolaris–H. argophyllus* as described by Burke et al. (2004).

Results

Intraspecific Genetic Mapping in Silverleaf Sunflower

Collectively, 1423 SSR, INDEL, and SSCP markers were screened for polymorphisms in the *H. argophyllus* mapping population. Of these, 227 amplified 299 polymorphic loci, where the *H. argophyllus* parent (ARG1805-2) was heterozygous ($A^{ARG}a^{ARG}$) and the NMS801 parent was homozygous ($A^{ANN}A^{ANN}$). Fifty DNA markers amplified two or more polymorphic loci each. The *H. argophyllus* genome was mapped by genotyping the polymorphic DNA marker loci in the interspecific F_1 testcross population (Fig. 1; Supplemental Fig. 1 and 2; http://www.sunflower.uga.edu/cmap). Segregation and recombination were tracked in the male (*H. argophyllus*) parent. When grouped using a minimum LOD threshold of 3.0, the 299 DNA marker loci assembled into 21 linkage groups of 4 to 30 loci each spanning 1370 cM with a mean density of one DNA marker locus per 4.6 cM. Of the 299 loci mapped in *H. argophyllus* in the present study, 200 were previously mapped in *H. annuus* and supplied a genome-wide framework of DNA marker loci for aligning and comparing *H. argophyllus* and *H. annuus* linkage groups (Tang et al., 2002; Yu et al., 2003; Radwan et al., 2008; http://www.sunflower.uga.edu/cmap/). The other 99 DNA marker loci had not been previously mapped in sunflower.

The 21 *H. argophyllus* linkage groups were aligned with 17 reference *H. annuus* linkage groups using 131 putative orthologous DNA marker loci among the 200 DNA marker loci mapped in both species (Supplemental Fig. 1). *Helianthus argophyllus* linkage groups are identified by the prefix ARG, whereas *H. annuus* linkage groups are identified by the prefix ANN (see Materials and Methods for linkage group naming conventions). *Helianthus annuus* linkage groups were well established, supported by genetic mapping of 1627 SSR, INDEL, SSCP, and SNP marker loci in several populations, and predicted to correspond to the 17 *H. annuus* chromosome pairs (Tang et al., 2002; Yu et al., 2003; Lai et al., 2005a; Radwan et al., 2008; http://www.sunflower.uga.edu/cmap/). Loci mapping to genomic locations in *H. argophyllus* incongruous with genomic locations in syntenic segments in *H. annuus* were assumed to be paralogous or erroneously genotyped. Therefore, they were identified as "rogue" DNA markers and were not used for synteny analysis (Burke et al., 2004; Lai et al., 2005b).

Macrosynteny between *H. annuus* and *H. argophyllus* identified putative subgroups of three of the 21 LOD-3.0 *H. argophyllus* linkage groups. When DNA marker loci were grouped and ordered using a minimum LOD threshold of 2.0, the 21 LOD-3.0 linkage groups merged into 18—ARG1A and ARG1B merged into a linkage group (ARG1) syntenic with ANN1, ARG9A, and ARG9B merged into a linkage group (ARG9) syntenic with ANN9, and ARG7-2 and ARG13-2 merged into a linkage group (ARG7/13-2) syntenic with ANN7 and ANN13 (Fig. 1; Supplemental Fig. 1 and 2). The 18 LOD-2.0 *H. argophyllus* linkage groups (ARG1, 2, 3, 4, 5, 6/15-1-INV, 6/15-2-INV, 7/13-1, 7/13-2, 8-INV, 9, 10, 11, 12A, 12B/16, 14-1, 14-2, and 17) were fully supported by macrosynteny with *H. annuus* linkage groups. Of the 18 LOD-2 linkage groups, 15 were predicted to identify 15 of the 17 pairs of chromosomes in *H. argophyllus* (ARG1, 2, 3, 4, 5, 6/15-1-INV, 6/15-2-INV, 7/13-1, 7/13-2, 8-INV, 9, 10, 11, 12B/16, and 17). The other three linkage groups were the shortest identified in *H. argophyllus*; ARG12A (29.5 cM), ARG14-1 (14.0 cM), and ARG14-2 (30.0 cM) (Supplemental Fig. 2). ARG12A and ARG12B/16 were predicted to be subgroups of a single linkage group (ARG12/16) from the colinearity of ARG12A and the ARG12B segment in ARG12B/16 to ANN12; however, ARG12A and ARG12B/16 did not merge at LOD 2.0. If these are subgroups of a single group, the ARG14-1 and

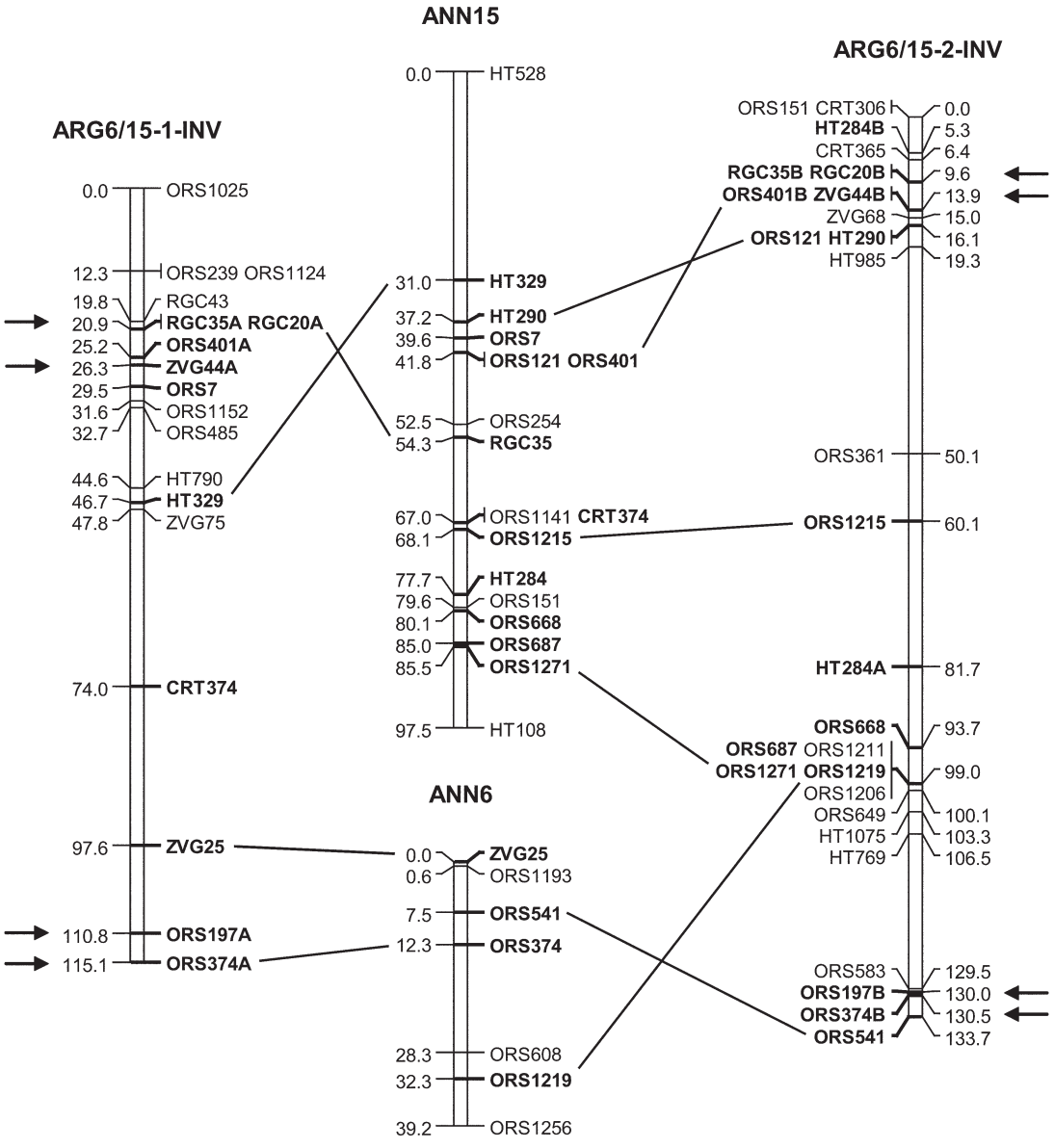


Figure 1. *H. annuus* and *H. argophyllus* linkage group alignments for chromosomes carrying rearrangements (translocations or inversions) or duplications. Overlapping duplicated segments on ANN8 and ANN17 are identified by red and yellow filled bars. Duplicated loci are identified by arrows.

ARG14-2 linkage groups might identify the other two pairs of chromosomes in *H. argophyllus*. This can only be settled by mapping additional DNA marker loci and denser genetic mapping in *H. argophyllus*.

Macrosynteny between the Common and Silverleaf Sunflower Genomes

Collectively, 28 colinear chromosomal segments were identified between *H. annuus* and *H. argophyllus* (Fig. 1; Supplemental Fig. 1). The 21 *H. argophyllus* linkage groups were syntenic with one or more segments of the 17 *H. annuus* linkage groups. We identified 10 colinear chromosomes (1, 2, 3, 4, 5, 9, 10, 11, 14, and 17), nine chromosomal rearrangements (five non-reciprocal translocations and four inversions), three putative segmental duplications, and two putative whole chromosome duplications (Fig. 1).

Two *H. argophyllus* chromosomes apparently arose by duplication (ARG6/15-1-INV and 6/15-2-INV, ARG7/13-1, and 7/13-2) and three *H. argophyllus* chromosomes apparently arose from the fusion of segmental duplications (ARG8, 14, and 17) (Fig. 1 and 2). Two *H. argophyllus* linkage groups (ARG14-1 and 14-2) were colinear with ANN14 and carried overlapping DNA marker loci and a single duplicated DNA marker locus (ORS434A and B) (Fig. 1). The upper 65.6 cM segment of ANN14 (HT534-ORS580) was not mapped in *H. argophyllus* because the sequences (DNA marker loci) were either not present or monomorphic. The overlapping and duplicated loci spanned a 34.5 cM segment (ORS580-HT319) in the lower half of ANN14. ARG14-1 and ARG14-2 may have arisen from the duplication of a common ancestral chromosome or

carry ancient duplications, as we are proposing for six other *H. argophyllus* chromosomes (ARG6/15-1-INV, ARG6/15-2-INV, ARG7/13-1, ARG7/13-2, ARG8-INV, and ARG17) (Fig. 1).

These segmental or whole chromosome duplications were identified primarily by mapping independent overlapping subsets of macrosyntenic DNA marker loci and secondarily by mapping paralogous (duplicated) loci.

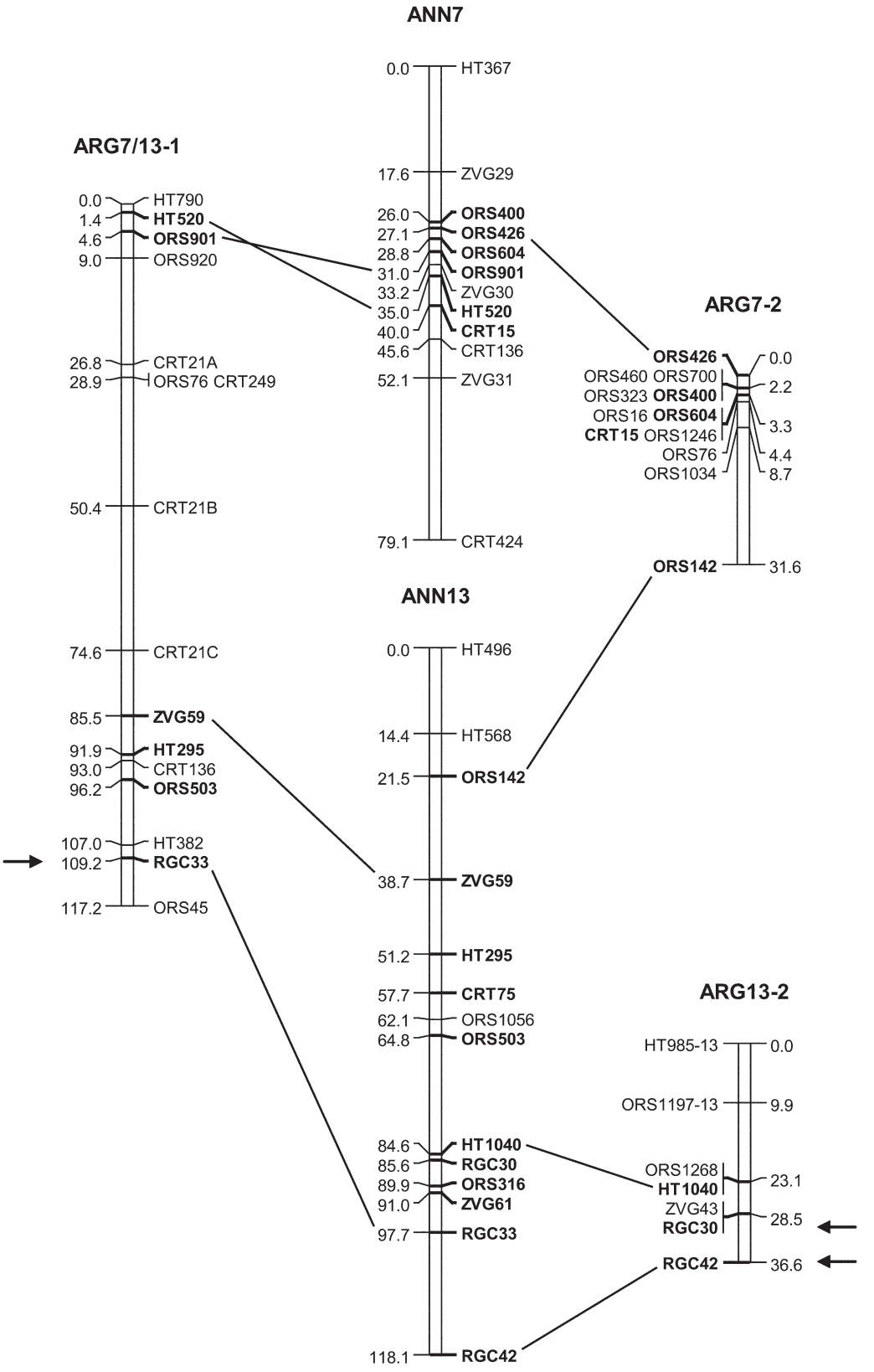


Figure 1. Continued.

The number of mapped paralogous loci was limited. We developed a hypothetical model of transitions leading to rearranged or duplicated chromosomes or chromosome segments to facilitate counting chromosomal breakages-fusions and to develop hypotheses for the evolution of *H. annuus* and *H. argophyllus* chromosomes (Fig. 2). Our model assumes the putative duplications arose subsequent to translocations and inversions. Under this assumption, the chromosomes of *H. annuus* and *H. argophyllus* are distinguished by three translocations ($n_T = 3$) instead of five and two inversions ($n_I = 2$), instead of four. Using $n_T + n_I = 5$ and a phylogenetic divergence time of 0.74 to 1.67 MYA (Strasburg and Rieseberg, unpublished data, 2009), the rate of karyotypic evolution (K) for these species was estimated to range from 1.5 to 3.4 chromosomal rearrangements/MYA. This should be a conservative estimate. If our model of karyotypic evolution is incorrect for these species (Fig. 2), and the observed translocations and inversions arose independently ($n_T + n_I = 9$), then K would range from 2.7 to 6.1 chromosomal rearrangements/MYA.

Architecture of Chromosome 5

ARG5 was identified as a colinear chromosome; however, an interstitial inversion may be present in the lower half of the chromosome spanning CRT376 and HT1021 (Fig. 1). The CRT376-HT1021 segment spanned 5.8 cM in *H. annuus* and 9.8 cM in *H. argophyllus*. The ordering difference was only supported by two loci, and the distances spanned by the putative inverted segment were borderline for unequivocally counting this as an inversion. Nevertheless, the presence of an interstitial inversion in ARG5 cannot be completely ruled out. ARG5 carries another short segment (HT321-ZVG19) with an inverted locus order in *H. argophyllus*. The HT321-ZVG19 segment spanned 2.1 cM in *H. annuus* and 4.3 cM in *H. argophyllus* and was probably caused by a locus ordering error.

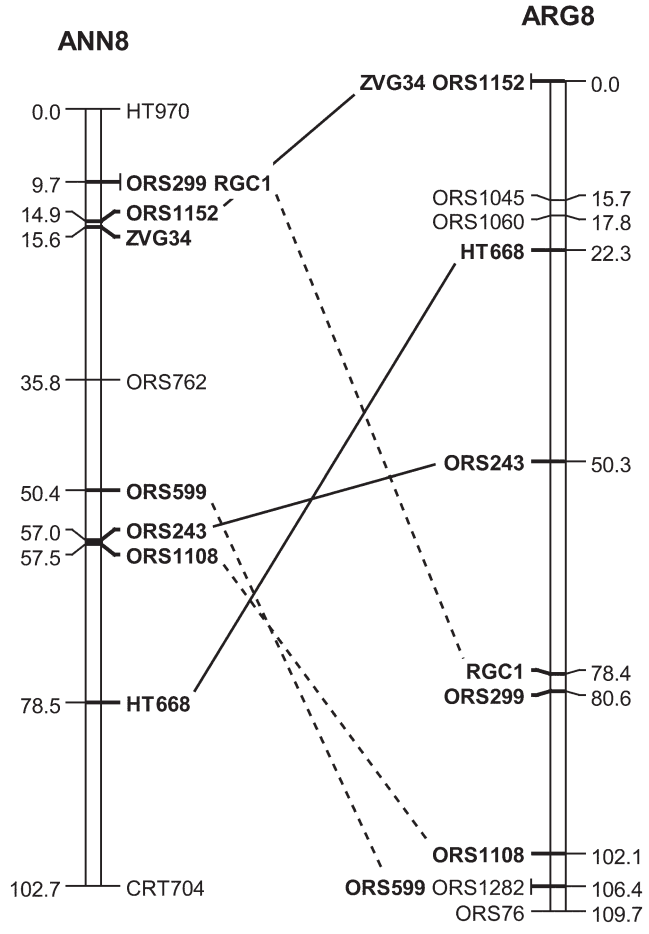
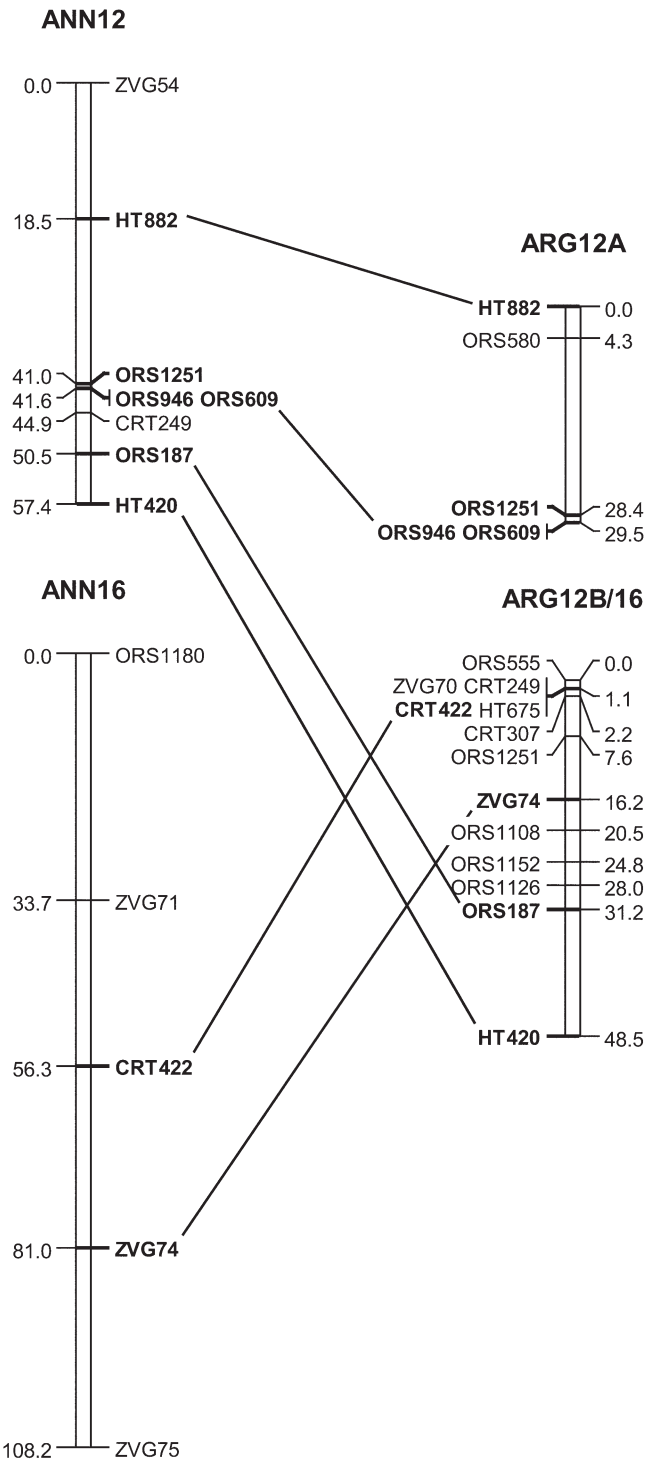


Figure 1. Continued.



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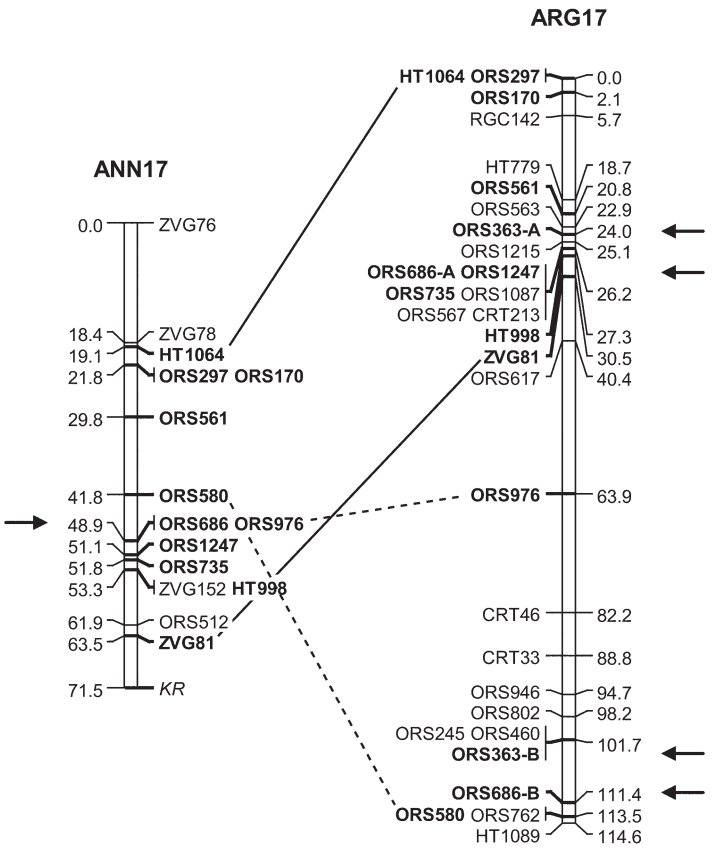

Figure 1. Continued.

Architecture of Chromosomes 6 and 15

ARG6/15-1-INV and ARG6/15-2-INV were colinear with each other, carried duplicated loci in the upper and lower ends of both chromosomes, inversions in the top segments of both chromosomes, and were apparently formed by the fusion of ANN6 and ANN15 (Fig. 1 and 2). The inversions spanned identical (overlapping) segments in both ARG6/15 chromosomes and were colinear with a single inverted ANN15 segment. HT329-RGC35A demarcated the 25.8 cM inversion in ARG6/15-1-INV, whereas ORS121-RGC35B demarcated the 6.5 cM inversion in ARG6/15-2-INV. The two ARG6/15 chromosomes carried overlapping subsets of independent DNA marker loci from ANN6 and ANN15, in addition to tightly linked clusters of paralogous (duplicated) loci in the upper and lower segments—ORS374A-ORS197A in the upper half and ZVG44A-ORS401A-RGC20A-RGC35A in the lower half of ARG6/15-1-INV and ORS374B-ORS197B in the upper half and ZVG44B-ORS401B-RGC20B-RGC35B in the lower half of ARG6/15-2-INV. Because *H. petiolaris* apparently shares the 6/15 translocation and *H. annuus* (ANN15) and *H. petiolaris* (PET6/15) lack the inversion in the segment syntenic to ANN15 in both ARG6/15 chromosomes in *H. argophyllus*, the inversion was inferred to postdate the fusion of ANN6 and ANN15 and predate the duplication of ARG6/15. ARG6/15-1-INV may carry a second inversion tracing to ANN6 spanning a 17.5 cM segment demarcated by ORS374A and ZVG25 (locus orders were reversed for ORS374A and ZVG25 in ARG6/15-1-INV and ANN6). This putative inversion, however, was only supported by a single pair of loci and may be a locus ordering error.

Architecture of Chromosomes 7 and 13

ARG7/13-1 and ARG7/13-2 were colinear with each other and with ANN7 and ANN13 segments, carried overlapping subsets of independent DNA marker loci, and were apparently formed by end-to-opposite end fusion of ANN7 and ANN13 (Fig. 1 and 2). The overlapping loci spanned a 14.0 cM segment (ORS400-CRT15) in the upper half of ANN7 and 33.5 cM segment (HT1040-RGC42) in the lower half of ANN13. ARG7/13-1 and ARG7/13-2 may have originated from the duplication of an ancestral chromosome.

Architecture of Chromosome 8

Overlapping subsets of DNA marker loci spanned the upper and lower segments of ARG8-INV (Fig. 1). Paralogous DNA marker loci were not mapped in the putative duplicated segments on ARG8-INV. The upper segment of ARG8-INV (ZVG34-HT668-ORS243) was 50.3 cM long and carried a putative interstitial inversion in the lower half of the segment (loci flanking the two colinear segments were ordered ZVG34-ORS243-HT668 in *H. annuus*). The inverted segment spanned 21.5 cM in *H. annuus* and 28.0 cM in *H. argophyllus*. The lower segment of ARG8 (RGC1-ORS1108-ORS599) was 28.0 cM long and carried a putative inversion in the lower 4.2 cM of the segment (loci flanking the two colinear segments were ordered RGC1-ORS599-ORS1108 in *H. annuus*). While loci flanking the putative inversion (ORS599-ORS1108) in the lower segment were only separated by 4.3 cM in *H. argophyllus* and 7.1 cM in *H. annuus*, the inverted segments on both the upper and lower segments of ARG8-INV traced to a single 17.2 cM segment in *H. annuus* delineated by overlapping subsets of independent DNA marker loci (ORS599-ORS243-ORS1108-HT668). The upper and lower segments of ARG8-INV were separated by a 28.1 cM long segment (ORS243-RGC1). ARG8-INV may have arisen by end-to-end fusion of two duplicated ancestral chromosomes in the ORS243-RGC1 segment, each carrying loci mapping to ANN8.

Architecture of Chromosome 17

The upper and lower segments of ARG17 were demarcated by overlapping subsets of independent DNA marker loci, in addition to two tightly linked pairs of duplicated DNA marker loci (ORS363A and B, ORS686A and B) (Fig. 1 and 2). The upper segment (HT1064-ZVG81) was 30.5 cM long. The lower segment (ORS976-ORS580) was 49.6 cM long and separated from the upper segment by a 33.4 cM segment (ZVG81-ORS976). ARG17 may have arisen from the end-to-opposite end fusion of two ancestral chromosomes colinear with ANN17. The overlapping ANN17 segments flank ZVG81 and

ORS976 (orders for overlapping and duplicated DNA marker loci were inverted in the lower segment ORS976-ORS580).

Chromosomal Rearrangements Shared by Multiple Sunflower Species

H. annuus, *H. argophyllus*, *H. petiolaris*, *H. anomalus*, *H. deserticola*, and *H. paradoxus* (Burke et al., 2004; Lai et al., 2005b; Fig. 1 and 3; Supplemental Fig. 1) were comparatively aligned to identify shared and unique chromosomal rearrangements. Only four chromosomes were colinear among the six species (1, 9, 10, and 17), and one of the four (ARG17) apparently carries duplicated ANN17 segments in *H. argophyllus* (Fig. 1, 2, and 3), and thus may not be strictly colinear. Because 6/15 fusions have been identified in *H. petiolaris* (PET6/15) and *H. argophyllus* (ARG6/15-1 and ARG6/15-2), ANN6 and ANN15 may have arisen by the breakage of an ancestral-6/15 chromosome, which may have predated the phylogenetic split of *H. annuus-H. petiolaris* 1.41 to 2.05 MYA (Strasburg and Rieseberg, unpublished data, 2009) and *H. annuus-H. argophyllus* 0.74 to 1.67 MYA (Strasburg and Rieseberg, unpublished data, 2009). ARG7/13-1 and ARG7/13-2 probably arose through the fusion of an ancestral-7 chromosome (intact and colinear in PET, ANO, DES, and PAR) and an ancestral-13 chromosome (rearranged in PET, ANO, DES, PAR, and ANN). Because ARG6/15-1 and ARG6/15-2, and ARG7/13-1 and ARG7/13-2 were colinear, the 6/15 and 7/13 fusions were hypothesized to predate the duplications (Fig. 1 and 2). Otherwise, the duplicated chromosomes arose through independent translocations, which seem improbable. We did not have information from the paralogous DNA marker loci needed to address this question.

By aligning *H. annuus*, *H. argophyllus*, and *H. petiolaris* linkage groups (Burke et al., 2004; Lai et al., 2005b; Fig. 1 and 3), the putative segmental duplications

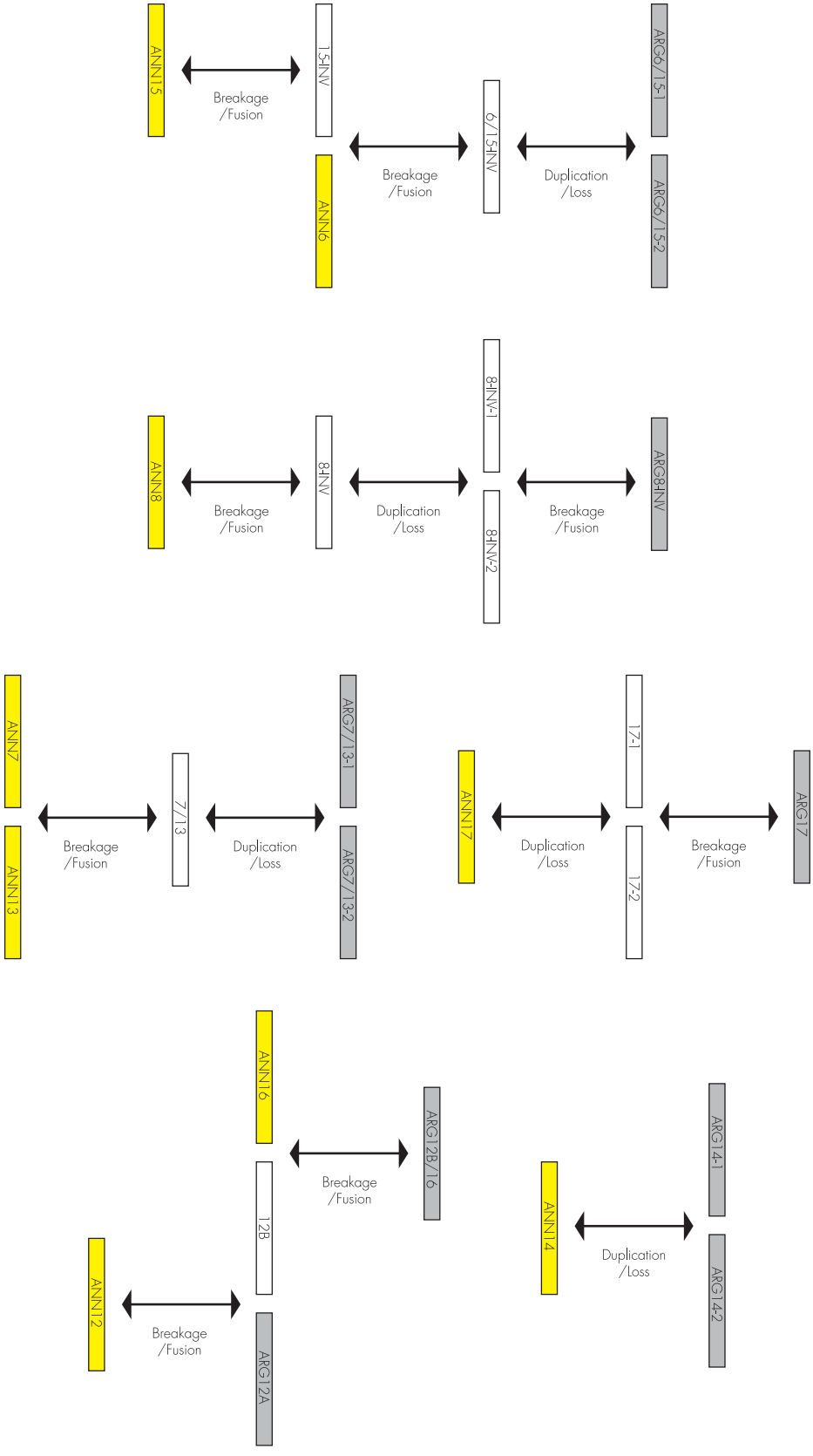

Figure 2. Chromosome breakages-fusions and putative duplications predicted from alignments of *H. annuus* and *H. argophyllus* linkage groups.

in ARG8 and ARG17 were discovered in *H. petiolaris*; hence, these duplications were predicted to predate the phylogenetic split between *H. petiolaris* and *H. annuus*. Using these linkage group alignments and updated estimates of phylogenetic divergence times (Strasburg and Rieseberg, unpublished data, 2009), rates of karyotypic evolution were estimated for these species and ranged from 2.7 to 3.9 chromosomal rearrangements/MYA for *H. annuus–H. petiolaris* and 2.2 to 3.2 chromosomal rearrangements/MYA for *H. argophyllus–H. petiolaris*.

The 6/15 and 7/13 duplications in *H. argophyllus* may have arisen subsequent to phylogenetic divergence of *H. annuus* and *H. argophyllus* 0.74 to 1.67 MYA (Strasburg and Rieseberg, unpublished data, 2009). ARG6/15-1 and ARG6/15-2 both carry inversions in segments colinear with ANN15, which implies this inversion predated the duplication. ANN8 and ARG8 originated from an ancestral-8 chromosome, although ARG8 apparently arose through the fusion of an ANN8 segmental duplication yielding two macrosyntenic segments in *H. argophyllus* which trace to a single macrosyntenic segment in *H. annuus* (Fig. 1 and 3). The interstitial inversions in the upper and lower segments of ARG8 were syntenic. Hence, the ARG8 inversions were predicted to predate the segmental duplication and fusion of the duplicated segments.

Discussion

Chromosomal Rearrangements and Karyotypic Evolution in Sunflower

Chromosomal rearrangements affect hybrid fertility, the transmission of alleles across species barriers, and the spectrum of genomic segments transmitted to interspecific hybrid offspring (Stebbins, 1971; Rieseberg, 2001; Levin, 2002). Chromosomal rearrangements identified between *H. annuus* and *H. argophyllus* shed further light on the complicated nature of karyotypic evolution in sunflower (Burke et al., 2004; Lai et al., 2005b). None of the translocations identified in *H. annuus* and *H. argophyllus* were reciprocal; hence, we found no evidence to support the hypothesis of two reciprocal translocations proposed by Chandler et al. (1986) and Quillet et al. (1995). Moreover, none of the translocations identified by comparative mapping in *H. annuus*, *H. argophyllus*, *H. petiolaris*, *H. anomalus*, *H. deserticola*, and *H. paradoxus* were reciprocal (Burke et al., 2004; Lai et al., 2005b), contrary to cytogenetic predictions (Chandler et al., 1986).

The putative duplications identified in *H. argophyllus* were primarily inferred by the colinearity of overlapping subsets of DNA marker loci, although paralogous DNA marker loci supported some of the inferences. The unidirectionality of the duplications, which were only identified in *H. argophyllus*, remains a puzzle. We did not identify duplicated chromosomes or chromosome segments in *H. annuus* tracing to a single chromosome or chromosome segment in *H. argophyllus*. The unidirectionality could be an artifact of the DNA markers used for macrosynteny analyses (Burke et al., 2004; Lai et al., 2005b; Fig. 1; Supplemental Fig. 1), which were developed from *H. annuus* DNA sequences and frequently amplify alleles from paralogous loci in other species (http://www.sunflower.uga.edu/cmap/). Comparative mapping of 474 SSR, INDEL, and SSCP markers identified 1124 loci in *H. annuus*, *H. argophyllus*, *H. petiolaris*, *H. anomalus*, *H. deserticola*, and *H. paradoxus*. Of these, 471 were paralogous DNA marker loci identified in wild species only (unpublished data, 2009).

So how do we account for the patterns of duplicated *H. annuus* segments in *H. argophyllus*? One explanation is that the duplicated segments represent remnants of ancient polyploidy (Sossey-Alaoui et al., 1998; Barker et al., 2008). However, this cannot be the full explanation because the main chromosomal duplications arose after the divergence of both species with *H. petiolaris* (Fig. 3). An alternative explanation, which minimizes the total number of rearrangements required to account for current synteny relationships, implies that most duplications arose within the *H. argophyllus* lineage and subsequent to translocations (Fig. 2). A third explanation is that while many of the duplications did arise after the divergence of *H. annuus* and *H. argophyllus*, they generally preceded the translocations and insertions associated with the same chromosomes. This hypothesis, while less parsimonious than the previous one, offers a possible mechanism for the establishment of rearrangements. Chromosome duplication reduces the initial underdominance of chromosomal rearrangements, thereby facilitating their establishment. Put another way, rearrangement-induced deletions are less likely to be lethal if the deleted genes have close paralogs elsewhere in the genome. However, duplicated genes tend to diverge over time, with one copy often lost or changing function (Lynch and Force, 2000). Because of this, rearrangements that were initially neutral (or even advantageous) should become strongly underdominant as redundancy is lost. Thorough analyses of paralogous DNA marker loci are needed to test different models of chromosome evolution in sunflower.

Silverleaf Sunflower as a Donor of Exotic Alleles for Common Sunflower Breeding

The macrosynteny analyses described here supply genomic blueprints for tracking the transfer of *H. argophyllus* alleles into *H. annuus* through MAS. Because the merit of a wild donor as a source of favorable alleles for enhancing complex traits normally cannot be ascertained from donor phenotypes per se, advanced backcross (ABC) QTL analysis and IL development are needed and should be powerful approaches for identifying and transferring favorable exotic alleles for complex traits in sunflower (Eshed and Zamir, 1995; Tanksley and Nelson, 1996; Zamir, 2001; Gur and Zamir, 2004). While numerous ILs have been developed for dominant race-specific disease resistance genes using *H. argophyllus* and other wild species as donors (Miller and Gulya, 1988; 1991; Seiler, 1991b; a), ILs have not been developed for genetically complex traits, and genome-wide

Figure 3. Circle diagram depicting macrosynteny among the genomes of three species of sunflower (inner circle = *H. annuus*, middle circle = *H. argophyllus*, and outer circle = *H. petiolaris*). Chromosomal rearrangements and putative segmental duplications are identified and numbered using *H. annuus* as the reference genome.

introgression line libraries have not been developed using *H. argophyllus* or any other wild species as a donor. The chromosomal rearrangements found in the genomes of *H. argophyllus* and other wild species of sunflower create complications for *H. annuus* IL development. Such complications do not arise in interspecific hybrids where the donor and recurrent parent genomes are nearly or completely colinear, although recombination is often suppressed in the introgressed segments (Zamir, 2001). Several *H. annuus* and *H. argophyllus* chromosomes are colinear (ARG1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 14, and 17) and should be straightforward targets for IL development, although three carry putative segmental duplications (ARG8, 14, and 17) (Fig. 1 and 3).

How these putative duplications affect chromosome pairing and recombination and the transmission of alleles in the duplicated segments is not known. This can be addressed by identifying the spectrum of recombinants produced among backcross and advanced generation progeny, developing a genome-wide library of ILs using *H. argophyllus* as the donor, and targeting duplicated segments when developing ILs through MAS.

Pyramiding Disease Resistance Genes in Sunflower

One aim of the present study was to identify chromosomal rearrangements or other factors affecting or impeding the transfer of disease resistance genes

between silverleaf and common sunflower. Pl_{ARG}, a silverleaf sunflower downy mildew *R*-gene introgressed into common sunflower, is found on linkage group 1 (Dussle et al., 2004), which appears to be completely colinear in *H. annuus* and *H. argophyllus* (Supplemental Fig. 1). The genomic location of Pl_{ARG} was identified by genetic mapping in a segregating population developed from a hybrid between HA342, a common sunflower inbred line, and ARG1575-2, an introgression line carrying Pl_{ARG} (Dussle et al., 2004). Several DNA marker loci had significantly distorted segregation ratios and recombination was suppressed in the introgressed segment, perhaps because of reduced homology between the *H. annuus* and *H. argophyllus* genomes. While the latter could be a factor, recombination in this segment was significantly lower in silverleaf than common sunflower in the present study (Supplemental Fig. 1) and could be the primary cause of suppressed recombination in segregating populations developed from crosses between elite *H. annuus* inbred lines and ILs carrying the Pl_{ARG} segment transmitted by the donor (*H. argophyllus*). Severe segregation distortion ($p < 0.001$) was found for all markers on ARG1 in the present study. The architecture of the chromosome segment could be dramatically different between *H. annuus* and *H. argophyllus*. The difference in recombination did not impede the introgression of the Pl_{ARG} segment into *H. annuus* (Dussle et al., 2004), but could complicate fine-scale mapping of Pl_{ARG} in intraspecific *H. argophyllus* and interspecific (*H. annuus* × *H. argophyllus*) populations.

The discovery of Pl_{ARG} opened up the possibility of pyramiding a minimum of three downy mildew *R*-genes in a single hybrid or cultivar, the others coming from large nucleotide binding site leucine rich repeat (NBS-LRR) *R*-gene clusters on ANN8 and ANN13 conferring resistance to multiple races—Pl_1, Pl_2, Pl_6, and Pl_7 on ANN8 and Pl_5 and Pl_8 on ANN13 (Bouzidi et al., 2002; Radwan et al., 2003, 2004, 2008; Slabaugh et al., 2003). Several NBS-LRR loci have been discovered in the segment harboring Pl_{ARG} and may belong to a family of NBS-LRR encoding downy mildew *R*-genes, one of which is Pl_{ARG} (Radwan et al., 2008). Several paralogous DNA marker loci have mapped to ANN8 and ANN13 segments spanning the NBS-LRR clusters (Tang et al., 2002, 2003; Slabaugh et al., 2003; Yu et al., 2003; http://www.sunflower.uga.edu/cmap/). The ANN8 and ANN13 clusters span 20 to 30 cM each and harbor numerous NBS-LRR loci in *H. annuus*. The ANN13 NBS-LRR cluster was discovered to be duplicated in *H. argophyllus* (Radwan et al., 2008; Fig. 1). NBS-LRR loci in the ANN13 cluster mapped to ARG7/13-1 and ARG7/13-2 (RGC30, 33, and 42). RGC1, a SSCP marker for one of several paralogous NBS-LRR loci in the ANN8 cluster (Slabaugh et al., 2003), mapped to the upper end of the lower duplication on ARG8. Thus far, NBS-LRR loci from the ANN8 cluster have not been mapped to the upper duplication on ARG8, but might be present and distal to ZVG43 and ORS1152 (Fig. 1). The diversity, duplications, and complexity of NBS-LRR clusters needs to be more deeply explored in *H. argophyllus* and other wild species used as *R*-gene donors in sunflower breeding. Sunflower hybrids resistant to multiple races of downy mildew can be developed by pyramiding genes within and among the three clusters of downy mildew *R*-genes identified so far in sunflower. Conceptually, pyramids of dominant downy mildew *R*-genes can be stacked in hybrids by fixing different *R*-genes in female and male inbred lines and targeting *R*-genes found in different (unlinked) clusters. The duplication of NBS-LRR clusters in wild species through chromosomal rearrangements and ancient polyploidy supply additional genetic diversity for developing disease-resistant hybrids in common sunflower.

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