COMPARATIVE MAPPING IN COMMON AND SILVER-LEAF SUNFLOWER

by

ADAM FRANCIS HEESACKER

(Under the Direction of Steven J. Knapp)

ABSTRACT

Several wild sunflower (*Helianthus*) species important sources of genetic diversity for enhancing the performance of cultivars and single-cross hybrids of common sunflower (*H. annuus* = ANN; 2n = 2x = 34). Silver-leaf sunflower (*H. argophyllus* = ARG; 2n = 2x = 34), which diverged 375,000 to 500,000 years before present from ANN, is a source of disease resistance alleles. We developed an ARG mapping population by crossing an outbred individual (ARG1805-1) to a nuclear male-sterile inbred line of ANN (NMS801), screened 1,417 previously mapped DNA markers, and mapped 299 polymorphic loci in the testcross hybrid mapping population (n = 94). Eighteen ARG linkage groups were aligned with ANN and chromosomal rearrangements were identified using 194 orthologous loci. We identified 11 colinear linkage groups, 2 syntenic chromosomes carrying segmental duplications and inversions, and 5 chromosomes carrying non-reciprocal translocations. The reduction in fertility and meiotic abnormalities observed in ANN x ARG hybrids are caused by 6 chromosomal rearrangements which could impede gene-flow.

INDEX WORDS: Comparative mapping, Introgression, Inversion, Sunflower, Synteny, Translocation

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DEDICATION

This thesis is dedicated to my family. My parents and instructors that helped my inquisitiveness grow, and my wife Sabra and my son James who helped keep me sane through the process.

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CHAPTER 1

INTRODUCTION

Purpose of Study

The sunflower genus *Helianthus* is native to North America. Common sunflower (*Helianthus annuus* L.) exists in both wild and domesticated forms. Its domesticated uses are as oilseed and confectionary crops and as a floral ornamental. The oilseed industry is the greatest factor to the monetary importance of sunflower. Due to the bottleneck of gene-flow that occurred during domestication, cultivated sunflowers lack the genetic diversity to adapt to present and emerging environmental (abiotic and biotic) challenges. For this reason, sunflower breeders screen for and introgress crop-beneficial traits from wild sources of diversity (wild *H. annuus* as well as other wild *Helianthus* species). The 13 wild annual species (which each have haploid chromosome numbers of n = 17) are most amenable to introgressive breeding. The species this research focuses on is the silver-leaf sunflower (*Helianthus argophyllus* T.&G.), which has been used for the introgression of insect and disease resistance, drought tolerance, and fertility restoration.

The goal of this research project is to gain a more thorough understanding of the genome of *Helianthus*, through comparative mapping of silver-leaf sunflower (*H. argophyllus* T.&G.) compared to common sunflower (*H. annuus* L.) which diverged 375,000 to 500,000 years ago (Rieseberg, 1991; Rieseberg et al., 1991). This research has practical applications, in-that introgressable segments from silver-leaf sunflower can be predicted and previous introgressions into sunflower can be elucidated. Chromosomal abnormalities (translocations and inversions)

have been shown to impede gene-flow between species, and are readily recognized through comparative mapping. A second outcome of this research is analysis of the rate of karyotypic evolution of the sunflower species thus far mapped. Analyses have previously been done in the prairie sunflower (*H. petiolaris* Nutt.) (Burke et al.2004), and the inter-specific hybrid species of *H. annuus* and *H. petiolaris* (the Pecos sunflower (*H. paradoxus* Heiser), desert sunflower (*H. deserticola* Heiser), and *H. anomalus* Blake (Lai et al. 2005b). A closer non-hybrid relative of cultivated sunflower, the silver-leaf sunflower (*H. argophyllus* T.&G.) is proposed as a counterbalance to the research done thus far, and can be used to date chromosomal changes along the *H. annuus/H. argophyllus* lineage.

State of the Public Sunflower Map:

Multiple sunflower linkage group nomenclatures have existed since the first complete linkage maps for sunflower were developed. Three main RFLP maps for sunflower were developed in France (Gentzbittel et al. 1995,1999), the USDA/ARS (Jan et al. 1998), and Advanta (Buenos Aires, Argentina; Berry et al. 1995,1997). These maps had the strength of reproducibility and thus the ability to standardize the sunflower linkage group nomenclature, though due to the sequences of the probes used being proprietary, exchange of information was limited. It took the development of the 1239 genomic simple sequence reapeats (ORS designation) and a collaboration with Advanta and the USDA to create the public maps (Gedil et al. 2001b; Tang et al. 2002; Yu et al. 2002,2003) that all have the same linkage group designations which represent the 17 chromosomes of sunflower. These maps were later merged into a high density composite map (Yu et al. 2003) from which a multiplex panel for high-throughput genotyping was derived (Tang et al. 2003). A standard linkage group nomenclature had been set.

Since that time, marker development and mapping have continued in sunflower. Of the 1239 original genomic SSRs, 722 of them have been mapped in sunflower. A total of 434 genomic SSR markers were developed by Pioneer Hi-Bred International (Johnston, IA) from sequences provided by the CARTISOL consortium, of which 162 have been mapped in at least one map of sunflower (SSL, CRT designation). The Compositae Genome Project (http://cgpdb.ucdavis.edu/database/Database_Description.html) produced 64,000 EST sequences that contained SNP, SSR, and indel polymorphisms. Two hundred and seventy three EST-SNP markers were developed and mapped using dHPLC on the public mapping RIL population RHA280xRHA801 (HT designation; Lai et al. 2005a). Of the EST-SSRs, 426 markers were developed of which 243 have been mapped (HT designation). Of the EST-indels, 39 were developed of which 29 have been mapped (HT designation). There has been development of indel, SSR, and SNP markers for 82 recently released, previously mapped cDNA RFLP probes from Advanta (ZVG designation). Approximately 200 SSCP markers were developed to assay the resistance genes of sunflower, of which 170 have subsequently been mapped (RGC designation). In all, these ~1500 markers represent a vast genomic resource for the mapping of common sunflower and comparative genomics with its wild relatives.

Accelerated Evolution in *Helianthus*:

The genus *Helianthus* has been the focus of multiple evolutionary studies due to its homoploid hybrid speciation events (Lai et al. 2005b) and its high rates of karyotypic evolution. Burke et al. (2004) showed that the prairie sunflower (*Helianthus petiolaris* Nutt.) compared to *H. annuus* had the highest rate of karyotypic evolution of any species studied thus far, a rate of 5.5-7.3 rearrangements per lineage per million years. The prairie sunflower population was made by making an intraspecific cross of two wild prairie sunflowers followed by an interspecific cross to

common sunflower to create interspecific F_1 hybrids for mapping (where segregation occurs only in the *H. petiolaris* genome). Initially this population was mapped with AFLPs and RAPDs (Rieseberg et al. 1995b). To facilitate comparison to common sunflower standard nomenclature, SSR markers developed by Yu et al. (2002, 2003) and Tang et al. (2002) were added. Mapping of the wild, hybrid, annual sunflower species (*H. anomalus, H. deserticola, H. paradoxus*) showed translocation activities not predicted by common introgressive hybridization of the parental species (*H. annuus, H. petiolaris*) (Lai et al. 2005b). The rate of karyotypic evolution for the hybrid species is greater than that of the parents due to these translocations, and the shorter amount of time from the hybrid speciation events. The mapping populations were made the same way as the previous *H. petiolaris* map, and the same RAPD, AFLP, and SSR markers were used. In both comparative mapping papers, a merged map of several published *H. annuus* linkage maps (Gedil et al. 2001a; Burke et al. 2002; Tang et al. 2002; Yu et al. 2003) was used for species comparisons. Through QTL analysis of pollen viability of interspecific F_1 hybrids, Lai et al. (2005b) were able to show that translocated linkage groups contained fertility QTL close to the location of the respective translocation points, and that translocations were a main cause of pollen sterility in F_1 hybrids. This observation mirrored the results seen by Quillet et al. (1995), though had more depth on the mapping of the species. Quillet et al. (1995) had only eight linkage groups compared to the full complement of 17 linkage groups seen in maps by Lai et al. (2005b).

Sunflower Crop Improvement using Silver-leaf Sunflower:

The silver-leaf sunflower has been a main source of genes for the improvement of cultivated sunflower and to that effect has contributed genes for fertility restoration to PET1 cytoplasm (Miller et al., 2002; Gustavo Abratti personal communication) and resistance to fungal pathogens. It has been reported to be a potential source of genes for drought resistance (Blanchet

and Gelfi, 1980; Seiler, 1983; Morizet et al. 1984; Baldini et al. 1992; Jamaux et al. 1997, El Midaoui et al. 2003). Jamaux et al. (1997) were able to capture the trait for osmotic adjustment in near isogenic lines (NILs) derived from an interspecific hybrid between silver-leaf and common sunflowers, though have not determined the genetic map location for this trait (which would help in efficient marker assisted selection).

In the case of introgressed disease resistance, silver-leaf sunflower has been a source of resistance to Phomopsis (*Diaporthe helianthi* Munt.-Cvet.), and downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni). Phomopsis stem canker is a disease that initially infects the leaves but travels to the stem through the petiole and creates a lesion at the axial thus making the plant more prone to lodging. Besnard et al. (1997) introgressed Phomopsis (*Diaporthe helianthi* Munt.-Cvet.) resistance into cultivated sunflower from silver-leaf sunflower, and were able to determine by RAPD-trait association that a minimum of two introgressed regions were required for explaining the level of resistance they found in their F_3 population. This information has not been utilized for marker assisted sunflower breeding due to genetic maps not being available.

Downy mildew (*Plasmopara halstedii*) is a fungal disease that reduces yield by suppressing plant growth, loss of photosynthesis due to chlorosis, and low seed fill (in some cases a seed head is never formed). There have been three cases of downy mildew resistance introgressed from silver-leaf sunflower. The first reported case of introgression of downy mildew resistance from *H. argophyllus* was of the resistance gene cluster containing Pl_8 (Miller and Gulya, 1988, 1991; Tan et al. 1992; Bert et al. 2001). This gene cluster has subsequently been mapped to linkage group 13 in *H. annuus* (Bouzidi et al. 2002; Slabaugh et al. 2003; Radwan et al. 2003,2004). The second instance of resistance gene introgression to this pathogen was identified by Dußle et al. (2004). Their group was able to place the new resistance gene they identified

(Pl_{Arg}) on LG1 in sunflower. This gene provides resistance to the more recently recognized strains of downy mildew. The order of SSR markers on LG1 was not colinear with previous maps of LG1 in *H. annuus* and suppressed recombination and segregation distortion were seen for the entire linkage group. Taken together, these factors led to the placement of Pl_{Arg} telomeric on LG1. The *H. argophyllus* map will be an important tool for mapping new RGCs (resistance gene candidates) and identifying the origins and genomic locations of other genes and genomic regions that can be and have been introgressed from *H. argophyllus* into *H. annuus*.

Previous Cytological Research of Silver-leaf Sunflower:

Multiple researchers have looked at meiotic abnormalities in pollen mother cells (PMCs) of interspecific hybrids of common and silver-leaf sunflower. The purposes of these studies were to determine change in chromosomal end arrangements (translocation) by multivalent formation and presence of paracentric inversions by the appearance of bridges and fragments at metaphase I through anaphase I of meiosis. The importance of these observations is that meiotic abnormalities in gamete formation lower the amount of viable pollen and lessen the chances of introgressing traits from wild sources. Another commonly recorded observation was the reduced pollen fertility of F_1 hybrids between *H. annuus* and *H. argophyllus*.

Heiser (1951) was the first to study the cytology of interspecific hybrids of common and silver-leaf sunflowers. Recorded pollen fertility ranged from 10 to 42%. Observed multivalent formations included one to three tetravalents (IV), and in very few cases hexavalents and octavalents were seen.

Chandler et al. (1986) performed interspecific crosses of multiple species of the annual sunflowers and recorded observations of F_1 pollen fertility, the presence of bridges-and-fragments and univalents, and multivalent configurations of PMCs. This group used PMCs of F_1 plants from

a single interspecific cross of a common sunflower and silver-leaf sunflower. Multivalent configurations were only called when it could be seen that crossing over had actually occurred (metaphase I and early anaphase I). This had the potential of excluding secondary associations of chromosomes seen in diakinesis (intraspecific homoeologous associations), though may have missed true interspecific associations. Observed pollen fertility was 12.7% and up to four univalents were seen in diakinesis and metaphase I PMCs. Multivalent configurations seen by Chandler et al. (1986) were one to two tetravalents. In these F_1 hybrids no bridges and fragments were seen. Comparison of maximal multivalent configurations of the species studied and pollen fertility led Chandler et al. (1986) to predict that only two reciprocal translocations separate the species of common and silver-leaf sunflower.

Quillet et al. (1995) performed a single interspecific cross between common and silver-leaf sunflower and observed pollen viability, univalent, and multivalent formation in two F_1 hybrids and 15 backcross individuals (recurrent common sunflower parent). Pollen viability of the F_1 hybrids was 24.9 and 27.2% while pollen viability of the BC_1s ranged from 27.2 to 93%. Up to four tetravalents were seen per PMC of the F1 hybrids and backcross progeny.

Atalagic et al. (2005) did the most complete job of describing the range of multivalent configurations presence in crosses between common and silver-leaf sunflowers. They made multiple interspecific crosses between the species and thus had a greater breadth of observations for gaining a true average of the translocations between species. They didn't do controlled crosses to assess intraspecific variation. Pollen viabilities ranged from 19.6 to 99%. The greater than 90% pollen viabilities have led to speculation that individuals showing the higher end of this range may have actually been backcrosses. In two of the three cases multivalents were seen which suggest that the chromosomes making up the multivalents don't confer a negative effect to pollen viability.

In one case anaphase I bridges were seen. The most common multivalent configurations were one to two tetravalents, others seen were two cells with a hexavalent and a tetravalent, and two cells containing three tetravalents. No crosses had consistently the same multivalent configurations as other crosses, suggesting that intraspecific chromosomal variation occurs in *H. argophyllus*.

Summary and Goals

Helianthus argophyllus is the closest living relative to *H. annuus*, and has been used extensively as a source of alleles for improvement of cultivated sunflower. Even though this species is commonly used in breeding, analysis of the chromosome rearrangements that differentiate these species has not been performed using comparative mapping. Therefore the goals of this thesis are:

1) Enhance the comparative mapping infrastructure in *Helianthus* by developing and mapping gene-based markers.
2) Predict the impact of chromosomal rearrangements on gene-flow between common and silver-leaf sunflower.
    a. Construct a 'complete' intraspecific map of *H. argophyllus.*
    b. Identify chromosomal rearrangements and barriers to gene-flow between the genomes of *H. annuus* and *H. argophyllus*.
    c. Estimate the rate of karyotypic evolution.
    d. Develop the framework for efficient marker-assisted selection in ANN x ARG hybrids and for producing a complete collection of wild introgression lines using ARG as the donor.

CHAPTER 2

COMPARATIVE MAPPING IN COMMON AND SILVER-LEAF SUNFLOWERS[1]

[1]Heesacker, A.F., R.L. Brunick, L.H. Rieseberg, and S.J. Knapp. To be submitted to *Genetics*.

ABSTRACT

Several wild species of *Helianthus* are sources of genetic diversity for enhancing agronomic and horticultural traits in common sunflower (*H. annuus* L. = ANN; 2n = 2x = 34). Silver-leaf sunflower (*H. argophyllus* L. = ARG; 2n = 2x = 34), the closest relative of common sunflower, diverged from *H. annuus* 375,000 to 500,000 years ago. *H. annuus* and *H. argophyllus* are inter-fertile, although hybrid fertility and viability are greatly reduced. *H. argophyllus* has been widely used as a source of novel disease resistance alleles and is a potentially important source of favorable alleles for enhancing drought tolerance, seed yield, and other complex traits in common sunflower. The goals of this study were to identify chromosomal rearrangements in the *H. argophyllus* genome with respect to the reference *H. annuus* genome through comparative genetic mapping. We developed an intraspecific *H. argophyllus* mapping population by crossing an outbred ARG individual (ARG1805-1) to a nuclear male-sterile inbred line of ANN (NMS801), screened 1,417 previously mapped DNA markers for polymorphisms between the parents, and mapped 299 polymorphic DNA marker loci in the hybrid mapping population (n = 94). The loci assembled into 21 linkage groups at LOD 3.0. *H. argophyllus* and *H. annuus* linkage groups were aligned using a genome-wide framework of 194 putative orthologous DNA marker loci selected from the 299 mapped DNA marker loci. We identified 11 colinear linkage groups, two syntenic chromosomes carrying segmental duplications and inversions, and five chromosomes carrying non-reciprocal translocations. The reduction in fertility and meiotic abnormalities observed in ANN x ARG hybrids are caused by a minimum of six chromosomal rearrangements. These rearrangements could impede interspecific gene-flow.

INTRODUCTION

Comparative mapping is a powerful tool for identifying syntenic and rearranged chromosomes, predicting the consequences of chromosomal rearrangements on interspecific gene flow, and developing strategies for efficiently introgressing wild species alleles into domesticated species through marker-assisted selection (MAS) (Rieseberg et al. 1995a,b; Noor et al. 2001; Zamir 2001; Burke et al. 2004; Gur and Zamir 2004; Koch and Keifer 2005; Lai et al. 2005b; Yogeeswaran et al. 2005). The advent of DNA markers and comparative mapping, initially in the Solanaceae and subsequently in the Poaceae, Cruciferae, and other plant families, facilitated cross-taxa synteny analyses, uncovered conserved (syntenic) gene orders among taxonomically divergent plant species (Bonierbale et al. 1988; Chao et al. 1989; Tanksley et al. 1988; Rieseberg et al. 1995b; Devos and Gale, 1997,2000; Lagercrantz, 1998; Wilson et al. 1999; Doganlar et al. 2002; Koch and Kiefer, 2005; Yogeeswaran et al. 2005), and facilitated the application of model species genomic resources for identifying and cloning loci underlying biologically and agriculturally important phenotypes across taxonomic boundaries (Gale and Devos 1998a,b; Paterson et al. 2000).

The development of genomic resources for common sunflower (*Helianthus annuus* L.; 2n = 2x = 34) has greatly facilitated comparative mapping in wild sunflower species and 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,b). The chromosomes of *H. annuus* and *H. petiolaris* Nutt. (2n = 2x = 34) and three *H. annuus* x *H. petiolaris* homoploid (2n = 2x = 34) hybrid species (*H. anomalus* Blake, *H. deserticola* Heiser, and *H. paradoxus* Heiser) have been comparatively mapped (Rieseberg 1995b; 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 (0.063-0.208 million years), a phenomenal number of chromosomal rearrangements were identified – 9 to 11 of the 17 linkage groups were non-syntenic in pairwise comparisons, and only four of the 17 linkage groups were colinear (Burke et al. 2004; Lai et al. 2005b). Burke et al. (2004) estimated the rate of karyotypic evolution in *H. annuus-H. petiolaris* at 5.5-7.3 chromosomal rearrangements per million years, higher than previously reported for other plant or animal taxa. Despite the presence of multiple chromosome rearrangements and greatly reduced interspecific hybrid fertility (Rieseberg et al. 1995b; Burke et al. 2004; Lai et al. 2005b), parent and hybrid species in the *H. annuus-H. petiolaris* complex are inter-fertile and supply genetic diversity for sunflower breeding (Heiser, 1951; Rogers et al. 1982; Chandler et al. 1986).

H. petiolaris and other wild sunflower species are rich and important sources of favorable alleles for broadening genetic diversity and enhancing agriculturally important traits in common sunflower. Silver-leaf sunflower (*Helianthus argophyllus* Torrey and Gray), the closest relative of common sunflower, has been widely used as a donor of favorable alleles for disease resistance and has been identified as a source of favorable alleles for drought tolerance and insect resistance (Satsyperov, 1916; Pustovoit et al., 1976; Pustovoit and Kroknin, 1978; Blanchet and Gelfi, 1980; Seiler, 1983, 1991a,b; Morizet et al., 1984; Kurnik and Walcz, 1985; Skoric, 1985; Rogers et al., 1987; Miller and Gulya, 1988, 1991; Baldini et al. 1992, 1997, 1998; Griveau et al., 1992; Tan et al., 1992; Quresh et al., 1993; Besnard et al., 1997; Jamaux et al., 1997; Degener et al., 1999a,b; Gulya, 2000; Viguié et al. 2000; Bert et al., 2001; Gedil et al. 2001a; Bouzidi et al., 2002; Langar et al., 2002; Miller et al., 2002; El Midaoui et al. 2003; Radwan et al. 2003, 2004; Slabaugh et al., 2003; Vear et al., 2003; Dußle et al., 2004). Historically, preceding the development of high-throughput DNA marker resources for sunflower (Tang et al. 2002, 2003; Yu et al. 2002,

2003), alleles from *H. argophyllus* and other wild species were introgressed into *H. annuus* by phenotypic selection and universal DNA landmarks were lacking for identifying and cross-referencing chromosome segments and loci carried by wild introgression lines (ILs). The earliest analysis of ILs carrying *H. argophyllus* introgressions was done using random amplified DNA polymorphism (RAPD) markers and identified three chromosome segments carrying quantitative trait loci (QTL) for Phomopsis stem canker (*Diaporthe helianthi* Munt-Cvet et al.) resistance (Besnard et al.1997). The locations of the QTL are not known.

Genetic mapping in *H. annuus* × *H. argophyllus* and *H. annuus* x *H. annuus* IL populations and graphical genotyping of *H. annuus* × *H. argophyllus* ILs have identified linkage groups and chromosome segments harboring downy mildew (*Plasmopara halstedii* (Farl.) Berl. & de Toni) resistance (*R*) genes introgressed from *H. argophyllus* (Slabaugh et al. 2003; Dußle et al., 2004). RHA340, an IL developed by selecting for resistance to downy mildew races 2, 3, and 4 (Miller and Gulya 1988, 1991), was graphically genotyped and discovered to carry an *H. argophyllus* introgression on linkage group 13 harboring one of two large clusters of downy mildew *R* genes identified in *H. annuus* (Gedil et al. 2001a; Bouzidi et al. 2002; Radwan et al. 2003, 2004, 2005; Slabaugh et al. 2003). The other large cluster is located on linkage group 8 (Gedil et al. 2001a; Slabaugh et al. 2003). ARG1575-2, an IL developed by selecting for resistance to downy mildew races 300, 700, 730, and 770, was discovered to carry an *H. argophyllus* introgression on linkage group 1 harboring a downy mildew resistance locus (Pl_{ARG}) (Duβle et al. 2004). Other than the downy mildew resistance loci found on linkage groups 1 and 13, the locations of other agriculturally important loci introgressed from *H. argophyllus* are not known.

Many of the resistance (*R*) genes found in plants encode proteins comprised of nucleotide binding (NB) domains and leucine-rich repeats (LRRs) (Staskawicz et al. 1995; Bent, 1996; Hammond-Kosack and Jones, 1997; Ellis et al. 2000). NB-LRR gene families are abundant in plant genomes and encode proteins necessary for triggering defense or 'guard' systems (Dangl and Jones, 2001; Meyers et al. 1999, 2003; Richly et al. 2002; Holt et al. 2003). Since the discovery of the role played by NB-LRR proteins in triggering disease resistance in plants, comparative genomic approaches have been used to isolate NB-LRR genes conferring resistance to a broad spectrum of bacterial, fungal, nematode, and viral pathogens (Bent, 1996; Kanazin et al. 1996; Leister et al. 1996, 1998; Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001; Meyers et al. 2003; Hulbert et al. 2001). Of the more than 40 *R* genes cloned from plants, 75% are members of NB-LRR gene families (Bent, 1996; Hammond-Kosack and Jones, 1997; Hulbert et al. 2001). The downy mildew *R* genes described in sunflower are dominant, race-specific, and typical of many other gene-for-gene resistances found in plants (Bent, 1996; Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001). Several of the downy mildew *R* genes found on linkage groups 8 and 13 encode NB-LRR proteins (Gedil et al. 2001a; Bouzidi et al. 2002; Radwan et al. 2003, 2004, 2005; Slabaugh et al. 2003). Only a few other NB-LRR gene families have been described and mapped in sunflower (Gedil et al. 2001a); however, Radwan et al. (in preparation) has since isolated more than 1,000 unique NB-LRR sequences, identified many new NB-LRR gene families, developed SSCP and other DNA markers for 195 NB-LRR loci, and genetically mapped 162 NB-LRR loci in common sunflower.

The discovery and transfer of disease resistance (*R*) genes has been the focus of most *H. annuus* × *H. argophyllus* breeding programs, primarily because disease resistance phenotypes can be directly ascertained in wild germplasm accessions and interspecific populations, despite the

segregation of wild species traits in the latter, e.g., branching, self-incompatibility, seed shattering, and seed dormancy (Burke et al. 2002; Gandhi et al. 2005; Tang et al. 2006). The discovery and transfer of favorable alleles for seed yield, drought tolerance, and other complex traits is more challenging, partly because the effects of favorable wild alleles are often obscured by the effects of unfavorable wild alleles, both in the wild parent and elite × wild segregating populations (Tanksley and Nelson 1996; Zamir 2001; Gur and Zamir 2004). Moreover, chromosomal rearrangements complicate breeding in interspecific populations by reducing hybrid fertility and recombination among rearranged chromosomes, problems normally circumvented by backcrossing to the elite parent (*H. annuus*) and selecting among backcross and advanced backcross progeny.

The analysis of karyotypic differences between common and silver-leaf sunflower has so far been limited to meiotic analyses (Heiser, 1951; Chandler et al. 1986; Quillet et al. 1995; Manjula et al. 1999; Atalagic et al., 2005). The genome of *H. argophyllus* has not been genetically mapped using intraspecific populations, a prerequisite for identifying chromosomal breakages and fusions against the framework of a reference genome (Rieseberg et al. 1995b). Common and silver-leaf sunflower shared a common ancestor 375,000 to 500,000 years ago (Rieseberg, 1991; Rieseberg et al., 1991). Despite the youth of both species, multiple rearrangements could be present in their genomes, particularly if the pattern found in other annual sunflower species holds (Rieseberg et al. 1995b; Burke et al. 2004; Lai et al. 2005b). The two species have identical chromosome numbers (2n = 2x = 34) and putatively identical karyotypes, although the resolution of previous karyotypic analyses were only sufficient for identifying gross morphological differences (Cuellar et al. 1995, 1999). Chromosome pairing abnormalities are frequently observed in pollen mother cells of *H. annuus* × *H. argophyllus* hybrids. While 17 bivalents were most commonly observed, ring-to-rod bivalent ratios were significantly lower and frequencies of

meiotic abnormalities were significantly greater in *H. annuus* × *H. argophyllus* hybrids than parents. The most prevalent meiotic abnormalities (identified at diakinesis), other than univalents (one to four), were 15 bivalents (II) + 1 tetravalent (IV), 13II + 2IV, 11II + 3IV, and 9II + 4IV (Heiser, 1951; Chandler et al. 1986; Quillet et al. 1995; Manjula et al. 1999; Atalagic et al. 2005). The chromosomes of *H. annuus* and *H. argophyllus* were predicted by Chandler et al. (1986) to carry two reciprocal translocations, a conclusion supported by Quillet et al. (1995). The presence of reciprocal translocations has not been substantiated by genetic mapping.

The spectrum of chromosomal segments recovered or lost in sunflower backcross breeding programs using *H. argophyllus* as the donor and the number, nature, and locations of chromosomal rearrangements between *H. annuus* and *H. argophyllus* are unknown. The goals of this study were to identify differences in chromosome architecture between *H. annuus* and *H. argophyllus*, gain a deeper understanding of the nature and rate of karyotypic evolution in sunflower, and develop 'genomic blueprints' to facilitate the efficient introgression of *H. argophyllus* alleles into *H. annuus* and development of introgression lines through MAS. This analysis was greatly facilitated by the development and genetic mapping of several hundred new EST-SSR and INDEL markers (Heesacker et al., in preparation) and by comparative mapping of several hundred SSR marker loci in wild and common sunflower (Tang et al. 2002; Yu et al. 2003; Burke et al., 2004; Lai et al., 2005b). When coupled with previously developed and mapped SSR, INDEL, and SNP markers (Tang et al. 2002; Yu et al. 2003; Lai et al. 2005a), our analysis drew upon more than 2,000 mapped DNA marker loci (Supplemental Figure 1; unpublished data).

MATERIALS AND METHODS

Development, growth, and DNA extraction of the hybrid population

A single plant of the nuclear male sterile (NMS) elite inbred oilseed line RHA801 (NMS801) was used as the female parent in a cross to ARG1805-2. ARG1805-2 was a seed of an out-bred self-incompatible individual of *H. argophyllus* ARG1805 (PI494571). The cross was made in Corvallis, OR during the summer of 2003. From the cross, 165 F_1 interspecific hybrid seeds were produced. Of those, 129 were germinated on blotter paper and surviving hybrids were potted and grown in the greenhouse. In all, 95 surviving plants provided leaf tissue for DNA extraction. DNA was isolated using a modified CTAB method (Webb and Knapp, 1990) and was quantified using the Synergy HT (BioTek Instruments Inc.; Winooski, VT, USA) as per recommendation. The mapping population was composed of 94 interspecific F_1 progeny of the NMS801 × ARG1805-2 cross.

PCR marker genotyping

All previously mapped (Burke et al. 2002, Tang et al. 2002; Yu et al. 2003; unpublished) PCR-based STS (sequence tagged site) markers (SSRs, indels, and SSCPs) were screened for polymorphisms on agarose and polyacrylamide gels using a bulk of 5 NMS801 elite inbred lines and a bulk of 20 F_1 progeny. PCR was performed using the 'Touchdown' PCR protocol (Don et al., 1991) and the method described by Tang et al. (2002) for all types of PCR markers. Fluorophore labeled SSRs and indels were multiplexed according to length and color (10 to 16 per well) and were genotyped using ABI Prism 377 polyacrylamide gels and GeneScan 2.1 and Genotyper 2.0 software (Applied Biosystems, Foster City, CA, USA). SSCP markers were multiplexed by amplicon size and screened on polyacrylamide gels as per Bertin et al. (2005). The PCR products were denatured and separated on SSCP gels using a mutation detection enhancement (MDE) gel

solution (Martins-Lopes et al. 2001). The gel mix was made in a 60-ml total volume containing a final concentration of 0.5× gel solution (Cambrex Bio-science Rockland, Rockland, Me.) and 0.6× TBE buffer and polymerized by the addition of 0.24 ml of 10% ammonium persulphate and 24 µl of tetramethyllenediamine (TEMED). Fragments were electrophoresed for 16 h at a constant power of 8 W at room temperature and then silver-stained as described by Bassam et al. (1991). Markers showing polymorphism were screened on a subpanel of 22 progeny to screen for segregation. Depending on the platform in which polymorphism was found (agarose, Genescan, or SSCP), markers were either run individually (agarose) or multiplexed according to size (Genescan, SSCP) and color (Genescan), to determine if segregation of alleles and therefore mappable polymorphism was available. Polymorphic markers were subsequently genotyped on the population of 94 interspecific F_1 progeny. Markers with agarose-based polymorphism were genotyped individually on agarose gels. Markers with SSCP-based polymorphism were multiplexed by size and genotyped on polyacrylamide gels. Markers with Genescan-based polymorphism were multiplexed by size and color (6 to 16 per well) and genotyped on the ABI 3730XL capillary system using Genemapper 3.5 software (Applied Biosystems, Foster City, CA, USA). Filter Set D and ROX 500 internal-lane standard were used for all ABI system reactions.

Genetic Mapping

The genetic map was constructed using the genotypes of 94 NMS801 × ARG1805-2 interspecific F_1 lines. Initial grouping was performed using G-MENDEL 3.0 (Holloway and Knapp, 1993), and backcross (BC) population set-up. This interspecific F_1 population has an expected segregation ratio of 1:1 which is equal to that of a BC population. Amplicon segregation data was recorded as 0 (absence of a band) or 1 (presence of a band), and both permutations (0 = A, 1 = H; 1 = A, 0 = H) were tested. Loci were grouped using likelihood odds (LOD) ratios with

LODs of >3.0 and a maximum recombination frequency threshold of 0.4. Due to the treatment, there were 2 copies of each linkage group (one positive and one in repulsion) with genotypic strings of both permutations. For each linkage group pair the permutation of all loci was made the same, and only one was kept. LODs < 3.0 were tried to add additional markers to the existing groups and to assess possible linkages below this pseudo-linkage threshold.

The detected linkage groups were entered into Mapmaker 3.0 (Lander et al., 1987) one at a time as F_2 backcross data. The Kosambi (1944) mapping function was used to calculate map distances (cM). Mapping was performed using order and ripple commands. Once the most likely order for a linkage group was determined, error detection was run and unproven double recombinants (most often genotyping errors) were removed from the dataset and the data was rerun using order and ripple commands. After multiple reiterations of this process, only one set of orders and distances had highest probability for each linkage group. This is a modified bin mapping method; bin mapping was first proposed in potato (Isidore et al., 2003). Chi-squared tests for segregation distortion were performed.

Linkage Group Naming and Nomenclature Rules

H. annuus linkage groups were numbered and oriented using standard linkage group nomenclature (Tang et al. 2002; Yu et al. 2003). ANN was added as a prefix to *H. annuus* linkage group numbers (1 = ANN1, 2 = ANN2, ..., 17 = ANN17). Several rules were applied when naming *H. agrophyllus* linkage groups and identifying colinear and rearranged chromosomal segments. First, *H. argophyllus* linkage groups were identified by the prefix ARG and numbered and oriented using the standard linkage group nomenclature for *H. annuus* (Tang et al. 2002; Yu et al. 2003). Second, colinear linkage groups were assigned identical numbers, e.g., ANN2 and ARG2. 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 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 (Lincoln and Lander, 1992; Hackett and Broadfoot, 2003) and were not counted as inversions. Seventh, inversions were only proposed when supported by two or more shared orthologous loci spanning segments longer than 4.0-6.0 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. Ninth, duplicated loci were identified by adding the suffix A, B, or C to the locus name.

RESULTS

Genetic Mapping in *H. argophyllus*

The *H. argophyllus* F_1 (interspecific hybrid testcross) mapping population was produced by pollinating a single male-sterile individual from a nuclear male-sterile *H. annuus* inbred line (NMS801) with pollen from a single male-fertile individual from an outbred *H. argophyllus* population (ARG1805-2). Heterozygous loci in the *H. argophyllus* parent segregated in the interspecific F_1 when the *H. annuus* parent was homozygous for one or neither of the alleles

transmitted by the *H. argophyllus* parent. By genotyping F_1 individuals, segregation and recombination were quantified in the male (*H. argophyllus*), but not the female (*H. annuus*), as in a backcross population (Rieseberg et al. 1995b).

We screened 1,423 SSR, INDEL, and SSCP markers for polymorphisms of these, 316 DNA markers failed to amplify alleles from one or both parents, whereas 573 DNA markers were polymorphic between NMS801 and a bulk of 20 NMS801 x ARG1805-2 F_1 individuals, and were subsequently screened for segregation among 22 randomly selected F_1 individuals. Of the 573 polymorphic DNA markers, 227 amplified 299 segregating loci in the mapping population and 50 of the 227 amplified two or more segregating loci. 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 (Figure 1), in addition to four tightly linked clusters of 2 to 3 markers. The 21 *H. argophyllus* linkage groups spanned 1,370 cM and had a mean density of 4.6 cM/locus.

The DNA markers genotyped and mapped in *H. argophyllus* were drawn from three sources: non-genic SSR markers (Tang et al. 2002, 2003; Yu et al. 2002, 2003), genic-INDEL markers developed for a previously mapped collection of cDNA-RFLP markers, and genic-SSR, INDEL, and SSCP markers (Heesacker et al., in preparation). Of the 299 DNA markers genotyped and mapped in *H. argophyllus*, 200 were previously mapped in *H. annuus*. These are identified as 'shared' DNA marker loci and supplied a genome-wide framework from which putative orthologous DNA marker loci were identified and selected for aligning and comparing *H. argophyllus* and *H. annuus* linkage groups (Burke et al. 2002; Tang et al. 2002, 2003; Yu et al. 2003; Supplemental Figure 1). The other 99 DNA marker loci had not been previously mapped in sunflower.

Genetic Mapping in *H. annuus*

The integrated *H. annuus* map assembled into 17 linkage groups corresponding to the 17 well established linkage groups. This map spanned 1,469.1 cM, and was constructed using 131 shared and 69 unique DNA marker loci (Figure 1). The latter were chosen to fill gaps between shared DNA marker loci, maintain the integrity of *H. annuus* linkage groups, and supply genome-wide coverage, as demarcated by DNA marker loci previously mapped in *H. annuus* (Burke et al. 2002; Tang et al. 2002, 2003; Yu et al. 2003; unpublished data). We used the 1,161-locus RHA280 × RHA801 RIL map as the framework map for producing a 204-locus integrated *H. annuus* map. The 1,161-locus map was stripped down to 156 essential, shared DNA marker loci. Then 33 DNA marker loci that were polymorphic in *H. argophyllus* were chosen from NMS373 × ANN1811 BC_1, PHA × PHB RIL, HA370 × HA372 F_2, and CMS89 × ANN1238 F_2 maps and integrated into the backbone map (Burke et al. 2002; Tang et al. 2002; Yu et al. 2003; unpublished Supplemental Figure 1). Non-framework DNA marker loci were either flanked by shared DNA marker loci or were telomeric (distal to a shared, telomeric DNA marker locus). Genetic distances and locus orders for non-framework DNA marker loci within an interval were drawn from a single non-framework map; non-framework DNA marker loci from multiple maps were not placed within an interval, but were placed in different intervals. The positions of non-framework DNA marker loci were found by interpolation.

Chromosomal Rearrangements Between the *H. annuus* and *H. argophyllus* Genomes

The DNA markers genotyped in *H. argophyllus* amplified orthologous loci or a combination of orthologous and paralogous loci or, rarely, paralogous loci only. We conservatively identified 143 putative orthologous DNA marker loci by comparing linkage group assignments and locus orders among *H. annuus* and *H. agrophyllus* linkage groups. Loci mapping

to linkage groups or positions in *H. argophyllus* incongruous with linkage groups and positions in syntenic (colinear) segments in *H. annuus* were presumed to either be paralogous or grouping or ordering errors (Burke et al. 2004; Lai et al. 2005b).

Orthologous DNA marker loci (shown in bold in Figure 1) were used to align and orient *H. annuus* and *H. agrophyllus* linkage groups and identify colinear segments and chromosomal breakages and fusions. We identified 28 colinear segments and eight chromosomal rearrangements (two inversions and four translocations) requiring a minimum of six chromosomal breakages-fusions. Two chromosomes apparently arose by non-disjuction or univalent addition (doubled or duplicated) (ARG6/15-1 and 6/15-2, and ARG7/13-1 and 7/13-2) and three chromosomes apparently arose from the fusion of doubled or duplicated chromosomes (ARG8, ARG17, ARG14-1, and 14-2) (Figures 1 and 2). Ten *H. annuus* and *H. agrophyllus* linkage groups were colinear (1, 2, 3, 4, 5, 9, 10, 11, 14, and 17). ARG1A and 1B were colinear with upper and lower segments of ANN1 and ARG9A and ARG9B were colinear with upper and lower segments of ANN9. When the minimum LOD threshold was lowered to 2.0, ARG1A and ARG1B merged into a single linkage group (ARG1) colinear with ANN1 and ARG9A and ARG9B merged into a single linkage group (ARG9) colinear with ANN9.

Two *H. argophyllus* linkage groups (ARG14-1and 14-2) were colinear with ANN14 and carried overlapping DNA marker loci and a single duplicated DNA marker locus (ORS434A and B) (Figure 1). The upper 65.6 cM segment of ANN14 (HT534-ORS580) was not mapped in *H. argophyllus* possibly due to the marker sequences not being present or not being polymorphic. 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, as perhaps have six other *H. argophyllus* chromosomes (ARG6/ 15-1-INV, ARG6/15-2-INV, ARG7/13-1, ARG7/13-2, ARG8-INV, and ARG17) (Figure 2). Putative duplicated chromosomes are identified by numerical suffixes (-1 and -2).

The upper and lower segments of ARG8-INV were spanned by overlapping subsets of independent DNA marker loci (duplicated DNA marker loci were not identified on ARG8-INV) (Figures 1 and 2). 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 found within 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 segment (ORS243-RGC1). ARG8-INV may have arisen by end-to-end fusion of two anciently duplicated ancestral chromosomes in the ORS243-RGC1 segment, each carrying loci mapping to ANN8.

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 and ORS686A and B) (Figures 1 and 2). The upper segment (HT1064-ZVG81) was 30.5 cM long. The lower segment (HT976-ORS580) was 49.6 cM long

and separated from the upper segment by a 33.4 cM segment (ZVG81-HT976). 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 HT976 (orders for overlapping and duplicated DNA marker loci were inverted in the lower segment HT976-ORS580).

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 (Figure 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 border-line for unequivocally identifying 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.

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 (Figures 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. Due to the facts that the 6/15 chromosomal configuration is shared with *H. petiolaris* and the fact that both *H. annuus* and *H. petiolaris* don't contain inversions on the 15 segment - the inversion undoubtedly postdated the fission of ANN6 and ANN15 and predated the ancient 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). The putative inversion, however, was only supported by a single locus (ZVG25) and may be a locus ordering error.

Three linkage groups carrying segments syntenic to ANN7 and ANN13 were found when using a minimum LOD of 3.0 (ARG7/13-1, ARG7-2, and ARG13-2) (Figures 1 and 2). When the LOD threshold was lowered to 2.0, ARG7-2 and ARG13-2 merged into a single linkage group (ARG7/13-2). ARG7/13-1 and 7/13-2 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 (Figure 1). 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.

Genome Coverage and *H. argophyllus* Linkage Groups

The 299 DNA marker loci mapped in *H. agrophyllus* were chosen to maximize genome coverage and spanned a minimum of 65.3% of the recombinational length of the *H. annuus* genome, as calculated by comparing distances spanned by shared DNA marker loci flanking the outer boundaries of each linkage group. The *H. argophyllus* map was only 109.2 cM shorter than the integrated *H. annuus* map. The mean difference in recombination frequency among shared

DNA marker intervals in *H. annuus* and *H. argophyllus* (0.223) was not significantly different (pair-wise *t*-test; $p = 0.63$) (Table 1). The 21 linkage groups presumably identify the 17 chromosomes found in *H. argophyllus*. Several telomeric *H. annuus* segments were not found in *H. argophyllus* (Table 2). The missing segments were either monomorphic for the DNA markers we screened or were part of the tightly linked groups of loci that didn't map to linkage groups at LOD threshold 3.

Despite screening 1,423 SSR, INDEL, or SSCP markers for polymorphisms, 17 *H. argophyllus* linkage groups could not be assembled at LOD 3.0, primarily because only 16% of the DNA markers were polymorphic and segregating in the mapping population. Burke et al. (2004) and Lai et al. (2005b) identified 17 linkage groups in four wild species (*H. petiolaris*, *H. deserticola*, *H. anomalus*, and *H. paradoxus*) by using a combination of SSR, RAPD, and AFLP markers and intraspecific hybrids between individuals from different wild populations. We used an intraspecific hybrid from a single wild population, a factor contributing to the paucity of DNA marker polymorphisms. While *H. argophyllus* germplasm has not been surveyed for DNA polymorphisms, greater diversity should be present among than within populations, and gaps in the present *H. argophyllus* map can almost certainly be closed by mapping in inter-population hybrids.

Three of the four tightly linked groups of loci accounted for the top of ANN2, the bottom of ANN11, and the bottom of ANN14. These groups only had linkage to their respective ARG linkage groups when the LOD threshold was lowered to 2, and were thus not added to the current maps. Six of the 21 LOD-3.0 linkage groups were identified as linkage subgroups in comparative alignments and assembled into three linkage groups at LOD-2.0 (ARG1, ARG9, and ARG7/13-1), leaving 18 linkage groups, of which a minimum of 15 were more or less complete and presumably

corresponded to 15 of the 17 *H. argophyllus* chromosomes (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). Of the three shortest linkage groups—ARG12A (29.5 cM), ARG14-1 (14.0 cM), or ARG14-2 (30.0 cM)—one could be a linkage subgroup of a chromosome carrying an unidentified translocation. This can only be resolved with additional genetic mapping. The number of chromosomal arrangements reported here (two inversions and four translocations) could underestimate the divergence between *H. annuus* and *H. argophyllus*.

Comparative Mapping and Karyotypic Evolution in *Helianthus*

Comparative mapping in intraspecific populations provides data for estimating of the number of chromosomal rearrangements and when coupled with cpDNA or other molecular estimates of species divergence times, the rates of karyotypic evolution (Lagercrantz 1998; Burke et al. 2004) can be calculated. While the genomes of six annual diploid species of sunflower have been comparatively mapped (Burke et al. 2004; Lai et al. 2005b; Figures 1 and 3), the rate of karyotypic evolution has only been estimated for two (*H. annuus*-*H. petiolaris*) (Burke et al. 2004). Using chromosomal rearrangements identified by comparative mapping of common orthologous DNA marker loci in each species (Burke et al. 2004; Lai et al. 2005b; Figures 1 and 3) and cpDNA estimates of divergence times (Rieseberg, 1991; Rieseberg et al. 1991; Schwazbach and Rieseberg, 2002; Welch and Rieseberg, 2002; Gross et al. 2003), we estimated rates of karyotypic evolution among three closely related species of sunflower (*H. annuus*, *H. petiolaris*, and *H. argophyllus*) and among the three homoploid hybrid species (*H. anomalus*, *H. deserticola*, and *H. paradoxus*) subsequent to interspecies (*H. annuus* x *H. petiolaris*) hybridization. The rates for the hybrid species were calculated using the number of chromosomal rearrangements estimated to have arisen subsequent to hybridization (Table 3). The rates for *H. annuus*-*H. argophyllus* (6.0-7.9

chromosomal rearrangements/MYA) and *H. argophyllus-H. petiolaris* (7.2-9.6) were virtually identical to the rate for *H. annuus-H. petiolaris* (5.4-7.1) (Burke et al. 2004; Table 3). The three hybrid species had slightly greater rates of karyotypic evolution than the parents. The rate for *B. rapa-B. oleracea*, the next fastest evolving pair of plant species compared so far, was 2.5 rearrangements/MYA, two- to nine-fold lower than *H. annuus-H. petiolaris* and other annual sunflower species (Lagercrantz 1998). Hence, karyotypes seem to have evolved more rapidly in sunflower than other plant and animal taxa.

DISCUSSION

Karyotypic Evolution in *Helianthus*

Chromosomal rearrangements have played an important role in plant evolution and speciation (Stebbins, 1971; Rieseberg, 2001; Levin, 2002). The structural differences found in *H. annuus* and *H. argophyllus* chromosomes shed additional light on the complicated and rapid nature of karyotypic evolution in the genus *Helianthus* (Table 3). These differences, when coupled with blueprints of the chromosomal rearrangements found in *H. annuus*, *H. petiolaris*, and *H. annuus* x *H. petiolaris* homoploid hybrid species (Rieseberg et al. 1995b; Burke et al. 2004; Lai et al. 2005b), facilitate historical reconstruction of the origin and persistence of chromosomal rearrangements in *H. annuus*, *H. argophyllus*, and *H. petiolaris* (Figure 3 and 4). When linkage groups found in the six previously mapped sunflower species were aligned, only four colinear chromosomes (1, 9, 10, and 17) were identified (Figure 3). One of the four (ARG17) apparently arose through the fusion of duplicated ANN17 segments and, consequently, was not strictly colinear with chromosome 17 in the other five species.

ANN6 and 15 arose by splitting a proto-6/15 chromosome which pre-dated the splits of *H. annuus-H. petiolaris* 0.75-1.0 million years before present (MYB) and *H. annuus-H. argophyllus* 0.38-0.5 MYB (proto-PET/ANN/ARG-6/15; Figure 4). The two ARG7/13 chromosomes arose through the fusion of a proto-ANN/ARG-7 chromosome (intact and colinear in PET, ANO, DES, and PAR) and a proto-ANN/ARG-13 chromosome (rearranged in PET, ANO, DES, PAR, and ANN). Because ARG6/15-1 and -2 and ARG7/13-1 and -2 were colinear in *H. argophyllus*, the 6/15 and 7/13 fusions must pre-date the duplications. The duplications of 6/15 and 7/13 must have arisen after the split between *H. annuus* and *H. argophyllus*. Both ARG6/15-1 and -2 also carry an inversion on the 15 segment which means that this inversion also predated the duplication of these chromosomes. ANN8 and ARG8 originated from a proto-ANN/ARG-8 chromosome, although ARG8 apparently arose through the fusion of ANN8 segmental or chromosomal duplications. The inversion on this chromosome is putatively duplicated at the bottom of this linkage group. If true, the timing of the inversion would predate the fusion of the duplicates.

A complete set of ancestral chromosomes were able to be predicted for the progenitor of *H. annuus* and *H. argophyllus*. Eleven chromosomes (1, 2, 3, 4, 5, 8, 9, 10, 11, 14, 17) were common to both *H. annuus* and *H. argophyllus*. Three of these chromosomes are duplicated within themselves (8, 14, 17) though the ancestral configuration of these chromosomes is expected to be like that of *H. annuus* due to its ability to survive with only one copy of these chromosomes. Duplications of 8 and 17 are seen in *H. petiolaris.* It is for this reason that the true ancestral state for these chromosomes can't be determined. Chromosomes 7, 13, and 6/15 are ancestral due to the reasons stated in the previous paragraph. This leaves the configurations of the chromosomes 12 and 16 in question. *H. argophyllus* and *H. petiolaris* do not share the same configurations for either of these chromosomes. Recent marker analysis shows that ancestral segment 16 is

triplicated in *H. petiolaris* (Figure 3), and that two of these are chromosomes that do not carry translocations. One of these is completely collinear with ANN16, and thus the untranslocated 16 and 12 are the ancestral configurations for these chromosomes. In all accounting the proto-ANN/ARG had 16 chromosomes and had a karyotype that mirrored *H. annuus* except that it had the ancestral 6/15 instead of 6 and 15.

Non-Additive Chromosomal and Segmental Duplications in *Helianthus*

Most eukaryotic genomes are of polyploid origin (Masterson, 1994; Spring, 1997; Wolfe and Shields, 1997; Matzke et al. 1999; Comai, 2000; Wendel, 2000). Sunflower is an ancient allopolyploid (paleopolyploid) with a physically large genome (3,000-3,400 Mb) and high frequency of duplicated loci (Berry et al. 1996; Gentzbittel et al. 1999; Tang et al. 2002, 2003; Baack et al. 2005). *H. annuus* and other 2n = 2x = 34 species of sunflower are hypothesized to have originated through hybridization of *Viguera* or other 2n = 2x = 16 species and spontaneous doubling of chromosomes in interspecific hybrids (Heiser and Smith, 1955; Jackson and Murray, 1983; Chandler, 1991; Schrader et al. 1997; Sossey-Alaoui et al. 1998; Schilling and Panero, 2002). More than half of the RFLP and SSR markers screened in *H. annuus* detect multiple loci (Berry et al. 1996; Gentzbittel et al. 1996; Tang et al. 2002, 2003). The ancient patterns of duplication found in the sunflower genome have not been described, and only a few segmental duplications have been identified (Slabaugh et al. 2003; Kolkman et al. 2004). The selection of low-copy RFLP probes for genetic mapping in sunflower hindered the discovery of paralogous loci and segmental duplications (Berry et al. 1996; Gentzbittel et al. 1996). DNA markers for SSRs primarily found in repetitive DNA sequences (e.g., retroelements) have identified several hundred loci in *Helianthus*, but have not shed light on ancient duplications either (Tang et al. 2002; Yu et al. 2003). While ancient duplications have not yet been identified in sunflower, several

chromosomal or segmental duplications were identified between *H. annuus* and *H. argophyllus* in the present study (Figure 1). The discovery of duplications produced through chromosomal rearrangements among recently diverged species (e.g., *H. annuus* and *H. argophyllus*) should be less difficult than identifying more ancient duplications produced by allopolyploidy, particularly because polyploid genomes are predicted to undergo rapid evolution subsequent to interspecific and intergeneric hybridization, e.g., loci are deleted and DNA sequences are lost, patterns of cytosine methylation and gene expression are altered, and the deleterious effects of translocations and other chromosomal rearrangements are buffered by gene redundancy (Matzke et al. 1999; Comai, 2000; Wendel, 2000; Liu et al. 2001; Shaked et al. 2001; Han et al. 2003; Langham et al. 2004; Pires et al. 2004a,b; Kovarik et al. 2005; Lukens et al. 2006).

Several of the architectural differences found in *H. annuus* and *H. argophyllus* chromosomes seem to have been produced by fusing duplicated chromosomes or chromosome segments (ARG8-INV, ARG14-1 and 14-2, and ARG17) or by duplicating chromosomes (ARG7/13-1 and 7/13-2 and ARG6/15-1-INV and 6/15-2-INV) (Figure 1). Chromosomal and segmental duplications were not identified in earlier comparative mapping analyses in *Helianthus* (Rieseberg et al. 1995b; Burke et al. 2004; Lai et al. 2005b), primarily because an insufficient number of duplicated loci and orthologous loci in *H. annuus* were mapped. Two or three *H. argophyllus* chromosomes (ARG8, 14, and 17) apparently arose through the fusion of duplicated chromosomes or chromosome segments end-to-end; ARG14-1 and 14-2 merged at LOD 2 but separated at LOD 3 (Figure 1). The duplications were identified by the presence of overlapping subsets of loci in *H. annuus* mapping to different segments or chromosomes in *H. argophyllus* and, less frequently, by the presence of paralogous loci in duplicated segments (ARG6/15-1-INV, 6/15-2-INV, 14, and 17, but not ARG7/13-1, 7/13-2, or 8) (Figures 1 and 5). DNA marker loci

mapped to putative duplicated segments were syntenic, apart from 'local' locus ordering errors, and inversions were preserved. The singletons mapped to one duplicated segment or the other could carry undetected paralogous loci in the opposite segment. The failure of many of the DNA markers genotyped in our study to identify paralogous loci in duplicated segments could have been because one locus was polymorphic and the other was monomorphic or because only one was amplified and the other was deleted or harbored deletions or other mutations.

The *H. annuus*-*H. argophyllus* duplications were non-additive, e.g., the fusion of two *H. annuus* chromosomes (ANN7 and ANN13) and subsequent duplication of the fused chromosome (ARG7/13-1 and 7/13-2) did not change chromosome number but doubled gene content in the duplicated segments in *H. argophyllus* (Figure 1). The functions of many of the genes found in duplicated segments produced by chromosomal rearrangements have presumably since been genetically and epigenetically altered (Matzke et al. 1999; Comai, 2000; Wendel, 2000; Liu et al. 2001; Shaked et al. 2001; Han et al. 2003; Pires et al. 2004a,b; Kovarik et al. 2005; Lukens et al. 2006). If the *H. annuus*-*H. argophyllus* duplications are *bona fide* then asymmetrical or non-additive gene duplications must be present in other sunflower species carrying chromosomal rearrangements and may be as important as polyploidy in the evolution of *Helianthus*, a genus where karyotypic evolution has played a particularly important role in speciation (Rieseberg et al. 1995b; Burke et al. 2004; Lai et al. 2005b).

Near-Isogenic and Segmental Introgression Line Development Using Silver-leaf Sunflower as a Donor

The comparative mapping analyses described here create genomic blueprints for designing breeding programs for efficiently transferring *H. argophyllus* alleles into *H. annuus* through marker-assisted selection, and for broadening the utility of *H. argophyllus* as a source of genetic

diversity for enhancing drought tolerance, seed yield, and other complex traits in common sunflower. Because the merits of wild species as donors of favorable alleles for enhancing complex traits usually cannot be ascertained from the phenotypes of the wild species donors *per se*, advanced backcross QTL discovery and segmental IL development strategies should have significant merit for identifying and transferring favorable alleles for complex traits in sunflower (Eshed and Zamir, 1995,1996; Tanksley and Nelson, 1996; Zamir, 2001; Morgante and Salamini, 2003; Gur and Zamir, 2004). Numerous early generation segmental ILs have been developed for monogenic disease resistance genes using *H. argophyllus* and other wild sunflower species as donors (Miller and Gulya, 1988, 1991; Seiler, 1991a,b). Far less has been done for complex traits (Besnard et al. 1997; Jamaux et al. 1997), complete segmental IL panels or "exotic genetic libraries" (Zamir, 2001) have not been developed for any wild donors, and wild species are underexploited as sources of genetic diversity for enhancing complex traits in sunflower.

The number of chromosomal rearrangements and unusual and apparently asymmetrical duplications found in *H. argophyllus* and other wild species create complications for IL development not found in species where donor and recurrent parent genomes are nearly or completely colinear, e.g., an apparently complete collection of segmental ILs has been developed for tomato (*Solanum lycopersicum* L.) using *S. pennellii* as the wild donor (Zamir, 2001), presumably because gene flow is not impeded by chromosomal rearrangements—the genomes of *S. lycopersicum* and *S. pennellii* have not been comparatively mapped using intraspecific populations). Several *H. annuus* and *H. argophyllus* chromosomes are colinear (1, 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 (8, 14, and 17) (Figure 1 and 3).

The presence of asymmetrical duplications in *H. argophyllus* and possibly other wild sunflower species raises several intriguing questions and could have important consequences for introgression line development. Can complete IL catalogs be developed for common sunflower using silver-leaf sunflower as the donor? When developing ILs for loci carried on duplicated *H. argophyllus* chromosomes or chromosomal segments, do the duplicated chromosomes or chromosomal segments pair with equal probability with the homologous ANN segment in interspecific hybrids? Do the ends of chromosomes produced by end-to-end fusions of duplications (ARG8, 14, and 17) pair with the ends of homologous ANN chromosomes (ANN8, 14, and 17), or is pairing offset? Do duplicated chromosomes produced by end-to-end fusions of two ANN chromosomes (ARG6/15-1 and -2 and ARG7/13-1 and -2) pair with the individual ANN chromosomes present in the fused ARG chromosomes, e.g., do ANN7 and ANN13 pair with ARG7/13-1 and ARG7/13-2 with equal probability in interspecific hybrids? Can two *H. annuus* ILs be developed for each duplicated segment found in *H. argophyllus*? If loci in duplicated *H. argophyllus* segments have undergone genetic and epigenetic changes since duplication (e.g., if paralogs have been deleted or silenced or evolved different patterns of expression), will *H. annuus* ILs carrying different duplicated *H. argophyllus* segments have asymmetrical gene compositions? Several of these questions can perhaps be answered by identifying, by graphical genotyping, the spectrum of recombinants produced among backcross progeny, and by developing ILs using *H. argophyllus* as the donor.

Pyramiding Disease Resistance Genes in Sunflower

One aim of our study was to identify chromosomal rearrangements impeding the transfer of disease resistance genes between silver-leaf and common sunflower. Pl_{ARG}, a downy mildew *R* gene transferred from silver-leaf into common sunflower, is found on a colinear chromosome

(ANN1-ARG1) (Duβle et al. 2004). Pl_{ARG} was identified and mapped by Duβle et al. (2004) in a segregating population developed from a hybrid between a common sunflower inbred line (HA342) and a segmental IL (ARG1575-2) carrying Pl_{ARG}. Several DNA marker loci had significantly distorted segregation ratios and recombination was suppressed in the introgressed segment (Duβle et al. 2004), presumably because of reduced homology, however, recombination in the introgressed segment was discovered to be significantly lower in silver-leaf than common sunflower (Figure 1) and could be the primary cause of heterogeneity of recombination in the Pl_{ARG} segment. Severe segregation distortion ($p<0.001$) was found for all markers on ARG1 in this mapping experiment. The architecture of the chromosome segment could be dramatically different between *H. annuus* and *H. argophyllus*, e.g., the segment ORS543 – ORS662 is genetically shorter (by 18.7-22.1 cM) and could be physically shorter in *H. argophyllus*. The difference in recombination did not impede the introgression of the Pl_{ARG} segment into *H. annuus* but could hinder high-resolution mapping of Pl_{ARG} in intraspecific *H. argophyllus* and interspecific 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 other two coming from large NB-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 2003, 2005; Slabaugh et al. 2003). The two clusters span 20 to 30 cM each and harbor multiple NB-LRR loci in *H. annuus*. By mapping NB-LRR loci, the ANN13 cluster was discovered to be duplicated and the ANN8 cluster may be duplicated in *H. argophyllus* (Figure 1). NB-LRR loci in the ANN13 cluster mapped to ARG7/13-1 and -2 (RGC30, 33, and 42). RGC1, an SSCP marker for one of several NB-LRR paralog loci in the ANN8 cluster (Slabaugh et al. 2003), mapped to the upper end of the lower

duplication on ARG8. Thus far, NB-LRR loci from the ANN8 cluster have not been mapped to the upper duplication on ARG8 but could be present and distal to ZVG43 and ORS1152 (Figure 1). Several paralogous DNA marker loci have mapped to ANN8 and 13 segments spanning the NB-LRR clusters (Tang et al. 2002, 2003; Yu et al. 2003; Slabaugh et al. 2003; unpublished data) and were presumed to identify a segmental duplication; however, the *R* genes found in the ANN8 cluster belong to the coiled coil (CC) or leucine zipper domain subfamily, whereas the *R* genes found in the ANN13 cluster belong to the Toll interleukin-1 receptor (TIR) subfamily (Pan et al. 2000; Michelmore and Meyers, 1998; Bouzidi et al. 2002; Meyers et al. 2003; Michelmore, 2003; Radwan et al. 2003, 2005). Pl_{ARG} apparently belongs to another NB-LRR cluster (Radwan et al. in preparation). The three downy mildew *R* gene clusters facilitate the development of inbreds and hybrids resistant to multiple races by pyramiding genes within and between clusters. Pyramids of dominant downy mildew *R* genes can be deployed in hybrids by selecting for different *R* genes in female and male inbred lines, targeting different *R* loci within clusters, and targeting different clusters. The duplication of NB-LRR clusters in wild species through chromosomal rearrangement and duplication supplies additional genetic diversity for battling disease resistance in sunflower.

Table 1: ANN-ARG Segment Recombination Differences

H. annuus **Linkage Group**	***H. argophyllus*** **Linkage Group**	**Segment**	***H. annuus*** **(cM)**	***H. argophyllus*** **(cM)**	**Difference (cM)**
1	1A	ORS1093-ORS959	28.5	45.2	-16.7
	1B	HT595-ZVG4	5.6	3.2	2.4
2	2	ORS1073-ORS342	45.9	46.7	-0.8
3	3	ORS1036-ORS822	78.6	57.4	21.2
4	4	ORS644-HT868	95.3	37.0	58.3
5	5	HT321-HT440	87.0	94.3	-7.3
6	6-1	ZVG25-ORS374	12.3	17.5	-5.2
	6-2	ORS541-ORS1219	24.8	34.7	-9.9
7	7-1	ORS901-HT520	4.0	3.2	0.8
	7-2	ORS400-CRT15	14.0	3.3	10.7
8	8-INV	ORS1152-HT668	63.6	50.3	13.3
		RGC1-ORS1108	47.8	28.0	19.8
9	9A	CRT108-HT1014	7.0	10.7	-3.7
	9B	ORS1265-ORS442	76.8	75.4	1.4
10	10	ORS595-ORS818	19.8	62.3	-42.5
11	11	ZVG49-ORS697	39.6	28.1	11.5
12	12A	HT882-ORS609	23.1	29.5	-6.4
	12B	ORS187-HT420	7.1	17.3	-10.2
13	13-1	ZVG59-RGC33	59.0	23.7	35.3
	13-2	HT1040-RGC42	33.5	13.5	20.0
14	14-1	ZVG62-HT319	25.8	10.7	15.1
	14-2	ORS580-ORS434	32.3	9.6	22.7
15	15-1-INV	HT329-CRT374	36.0	25.8	10.2
	15-2-INV	HT290-ORS1271	48.3	89.4	-41.1
16	16	CRT422-ZVG74	24.7	15.1	9.6
17	17	HT1064-ZVG81	44.4	30.5	13.9
		ORS580-ZVG81	21.7	83.0	-61.3
Total			1006.5	945.4	61.1

Table 2: *H. annuus* Segments Not Mapped in *H. argophyllus*

Linkage group	Segment	Length (cM)	Location Telomeric
ANN2	HT63 - ORS1073	10.9	Top
ANN3	ORS822 - HT806	9.1	Bottom
ANN4	HT868 - HT327	7.5	Bottom
ANN6	ORS1219 – ORS1256	6.9	Bottom
ANN7	CRT15 - CRT424	39.1	Bottom
ANN8	HT970 – RGC1	9.7	Top
ANN8	HT668 - CRT704	24.2	Bottom
ANN9	HT379 - CRT108	38.8	Top
ANN10	ZVG43 - ORS591	45.3	Top
ANN10	ORS818 - HT601	14.1	Bottom
ANN11	HT153 - ZVG49	9.4	Top
ANN11	ORS679 - HT293	31.5	Bottom
ANN12	ZVG54 - HT882	18.5	Top
ANN13	HT496 - ORS142	21.8	Top
ANN14	HT534 - ORS1079	63	Top
ANN14	HT319 – RGC64	26	Bottom
ANN15	HT528 - HT329	31	Top
ANN15	ORS1271 - HT108	12	Bottom
ANN16	ORS1180 – CRT422	56.3	Top
ANN16	ZVG74 - ZVG75	27.2	Bottom
ANN17	HT1064 – ZVG76	19.1	Top
ANN17	ZVG81 – KR	8	Bottom

Table 3: Rate of Karyotypic Evolution

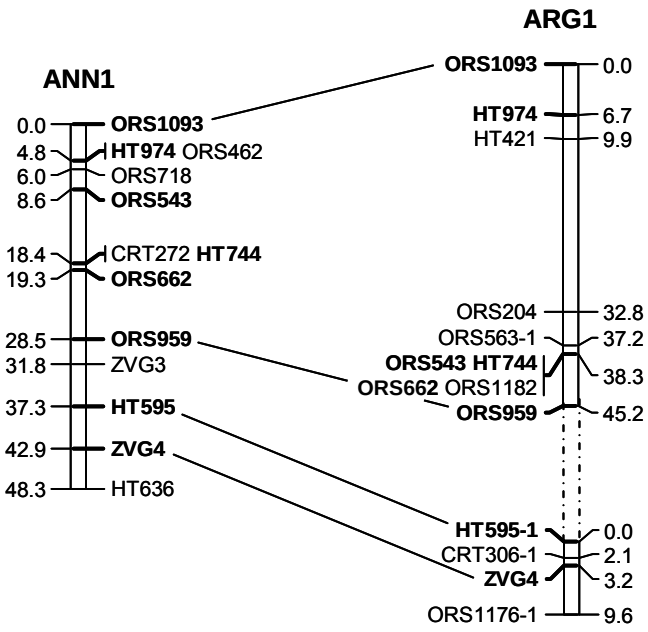
Species Compared	**cpDNA Diversity (%)[a]**	**Number of translocations (T) and inversions (I)[b]**	**Divergence time (million years)**	**Number of translocations subsequent to hybrid speciation**	**Rate of karyotypic evolution[c]**
H. annuus- *H. argophyllus*	0.06	4 T + 2 I	0.375 - 0.500	NA	6.0-7.9
H. annuus- *H. petiolaris*	0.12	8 T + 3 I	0.750 - 1.000	NA	5.5-7.3
H. petiolaris- *H. argophyllus*	0.10	8 T + 4 I	0.625 - 0.833	NA	7.2-9.6
(*H. annuus x* *H. petiolaris*)- *H. anomalus*	0.01-0.11	8 T/11 T	0.116 - 0.160	5 T	10.4-14.4
(*H. annuus x* *H. petiolaris*)- *H. deserticola*	0.05-0.15	5 T/8 T	0.063 - 0.170	2 T	3.9-10.6
(*H. annuus x* *H. petiolaris*)- *H. paradoxus*	0.02-0.10	7 T/11 T	0.075 - 0.208	7 T	11.2-31.1

a Statistics from Rieseberg et al. (1991)

b Breakage-fusion events

c Rate of karyotypic evolution calculated as (number of breakage-fusion events)/(number of species compared × divergence time in millions of years)

Figure 1. Comparative alignments of *H. annuus* (ANN) and *H. argophyllus* (ARG) linkage groups. Shared orthologous DNA marker loci are shown in bold and identify the boundaries of colinear segments. ORS and CRT prefixes identify non-genic SSR markers, the ZVG prefix identifies genic-INDEL or SNP markers, the HT prefix identifies genic-SSR or INDEL markers, and the RGC prefix identifies SSCP markers for resistance gene candidates. Solid and dashed lines identify duplicated segments or linkage groups in *H. argophyllus* tracing to a single segment or linkage group in *H. annuus*. Duplicated loci are identified by arrows. LOD = 2 linkage group merges are shown by dashed linkage group segments.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6.0 ORS718
8.6 ORS543
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19.3 ORS662
28.5 ORS959
31.8 ZVG3
37.3 HT595
42.9 ZVG4
48.3 HT636
ARG1
ORS1093 0.0
HT974 6.7
HT421 9.9
ORS204 32.8
ORS563-1 37.2
ORS543 HT744 38.3
ORS662 ORS1182
ORS959 45.2
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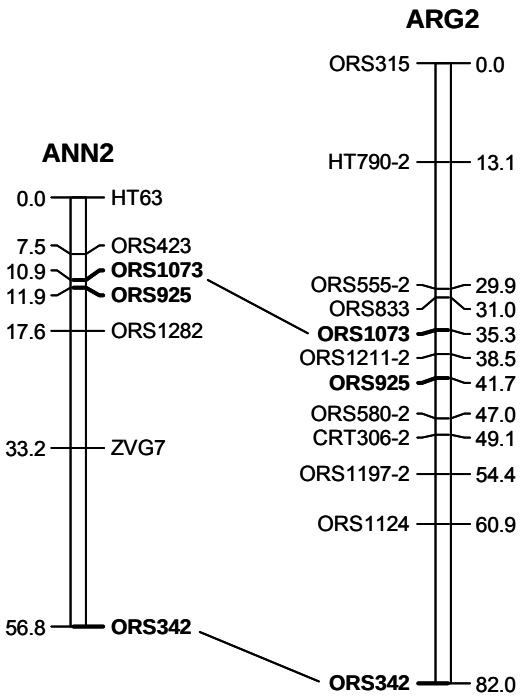
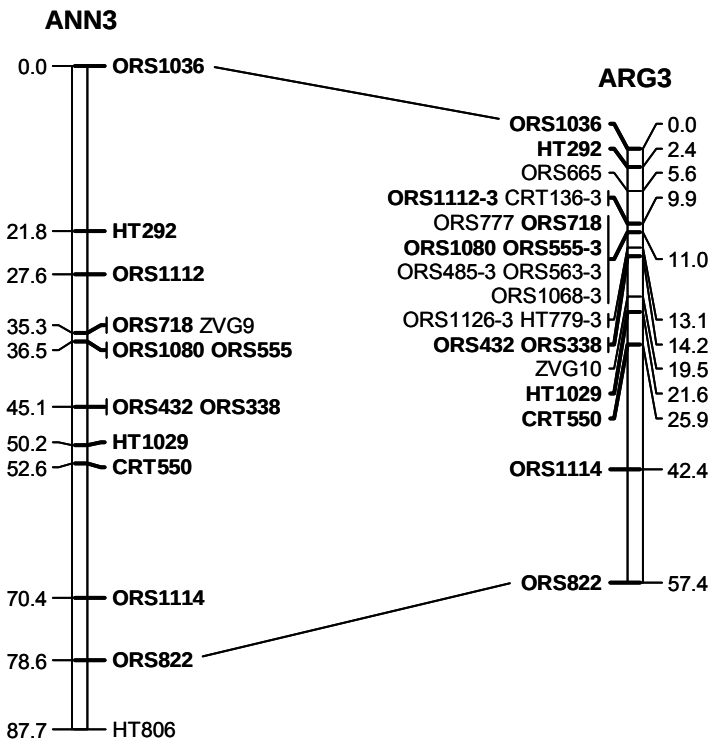
CRT306-1 2.1
ZVG4 3.2
ORS1176-1 9.6


ANN2
0.0 HT63
7.5 ORS423
10.9 ORS1073
11.9 ORS925
17.6 ORS1282
33.2 ZVG7
56.8 ORS342
ARG2
ORS315 0.0
HT790-2 13.1
ORS555-2 29.9
ORS833 31.0
ORS1073 35.3
ORS1211-2 38.5
ORS925 41.7
ORS580-2 47.0
CRT306-2 49.1
ORS1197-2 54.4
ORS1124 60.9
ORS342 82.0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ORS1036
21.8
HT292
27.6
ORS1112
35.3
ORS718 ZVG9
36.5
ORS1080 ORS555
45.1
ORS432 ORS338
50.2
HT1029
52.6
CRT550
70.4
ORS1114
78.6
ORS822
87.7
HT806
ARG3
ORS1036
HT292
ORS665
ORS1112-3 CRT136-3
ORS777 ORS718
ORS1080 ORS555-3
ORS485-3 ORS563-3
ORS1068-3
ORS1126-3 HT779-3
ORS432 ORS338
ZVG10
HT1029
CRT550
ORS1114
ORS822
0.0
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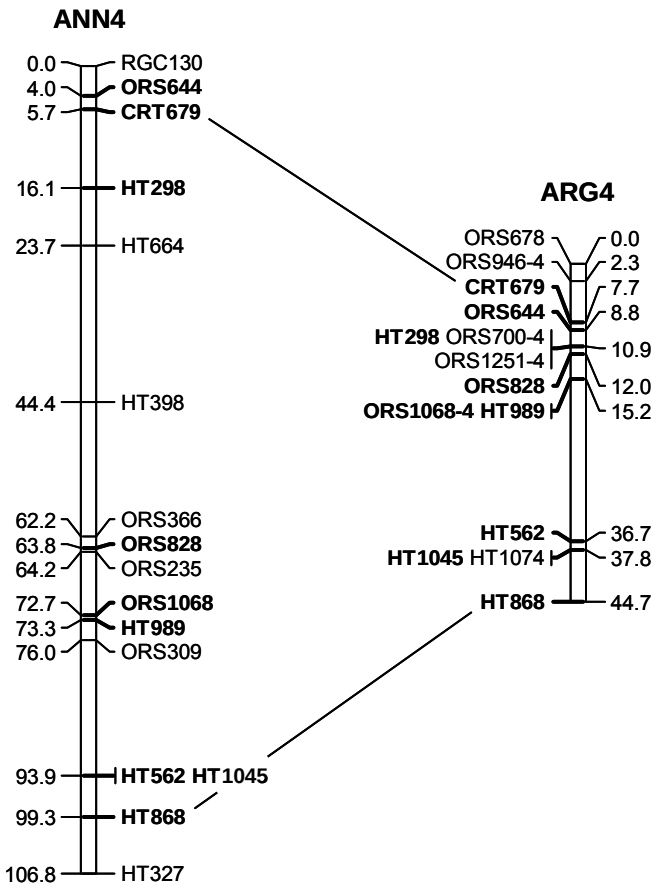
5.6
9.9
11.0
13.1
14.2
19.5
21.6
25.9
42.4
57.4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0.0 RGC130
4.0 ORS644
5.7 CRT679
16.1 HT298
23.7 HT664
44.4 HT398
62.2 ORS366
63.8 ORS828
64.2 ORS235
72.7 ORS1068
73.3 HT989
76.0 ORS309
93.9 HT562 HT1045
99.3 HT868
106.8 HT327
ARG4
ORS678 0.0
ORS946-4 2.3
CRT679 7.7
ORS644 8.8
HT298 ORS700-4
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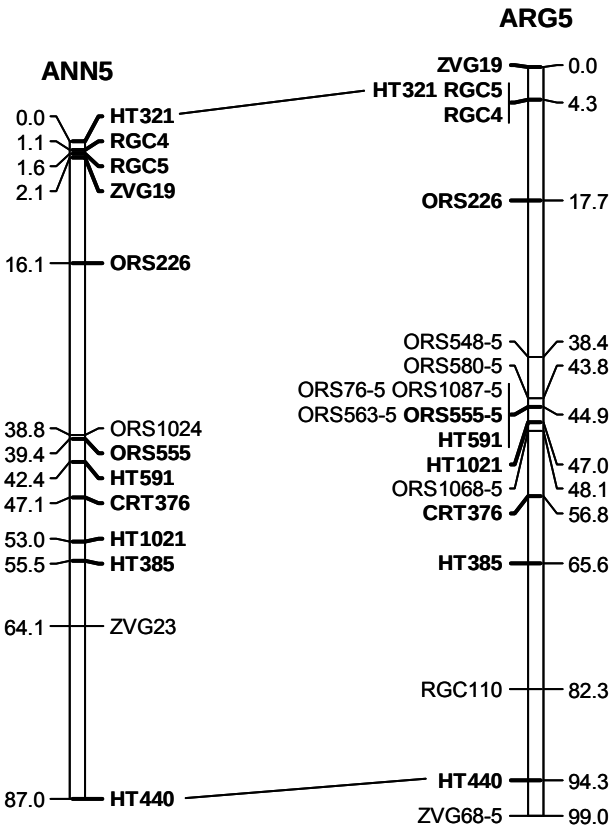
ORS828 12.0
ORS1068-4 HT989 15.2
HT562 36.7
HT1045 HT1074 37.8
HT868 44.7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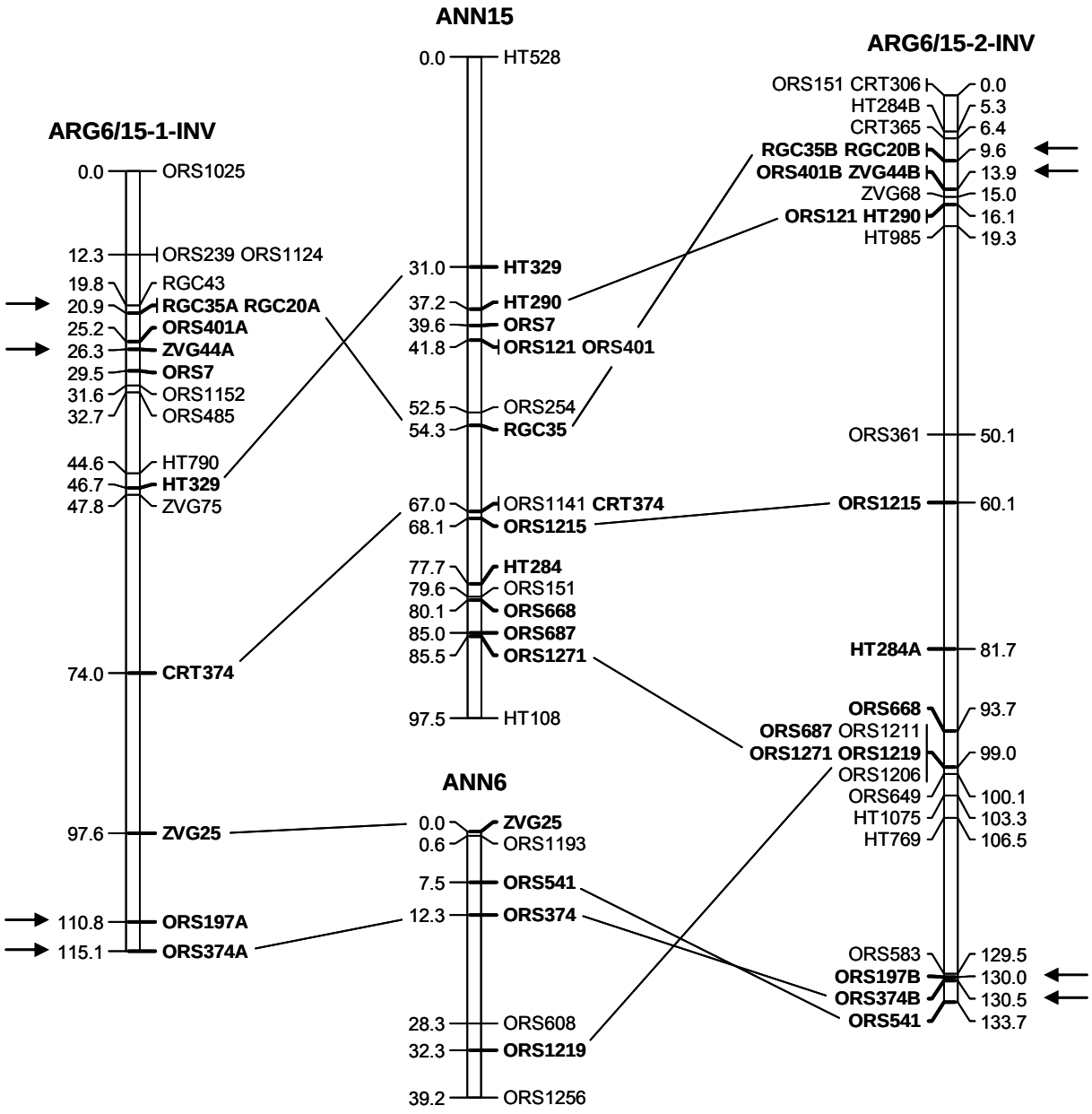
1.1 RGC4
1.6 RGC5
2.1 ZVG19
16.1 ORS226
38.8 ORS1024
39.4 ORS555
42.4 HT591
47.1 CRT376
53.0 HT1021
55.5 HT385
64.1 ZVG23
87.0 HT440
ARG5
ZVG19 0.0
HT321 RGC5 4.3
RGC4
ORS226 17.7
ORS548-5 38.4
ORS580-5 43.8
ORS76-5 ORS1087-5
ORS563-5 ORS555-5 44.9
HT591
HT1021 47.0
ORS1068-5 48.1
CRT376 56.8
HT385 65.6
RGC110 82.3
HT440 94.3
ZVG68-5 99.0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31.0 HT329
37.2 HT290
39.6 ORS7
41.8 ORS121 ORS401
52.5 ORS254
54.3 RGC35
67.0 ORS1141 CRT374
68.1 ORS1215
77.7 HT284
79.6 ORS151
80.1 ORS668
85.0 ORS687
85.5 ORS1271
97.5 HT108
ARG6/15-1-INV
0.0 ORS1025
12.3 ORS239 ORS1124
19.8 RGC43
20.9 RGC35A RGC20A
25.2 ORS401A
26.3 ZVG44A
29.5 ORS7
31.6 ORS1152
32.7 ORS485
44.6 HT790
46.7 HT329
47.8 ZVG75
74.0 CRT374
97.6 ZVG25
110.8 ORS197A
115.1 ORS374A
ARG6/15-2-INV
ORS151 CRT306 0.0
HT284B 5.3
CRT365 6.4
RGC35B RGC20B 9.6
ORS401B ZVG44B 13.9
ZVG68 15.0
ORS121 HT290 16.1
HT985 19.3
ORS361 50.1
ORS1215 60.1
HT284A 81.7
ORS668 93.7
ORS687 ORS1211
ORS1271 ORS1219 99.0
ORS1206
ORS649 100.1
HT1075 103.3
HT769 106.5
ORS583 129.5
ORS197B 130.0
ORS374B 130.5
ORS541 133.7
ANN6
0.0 ZVG25
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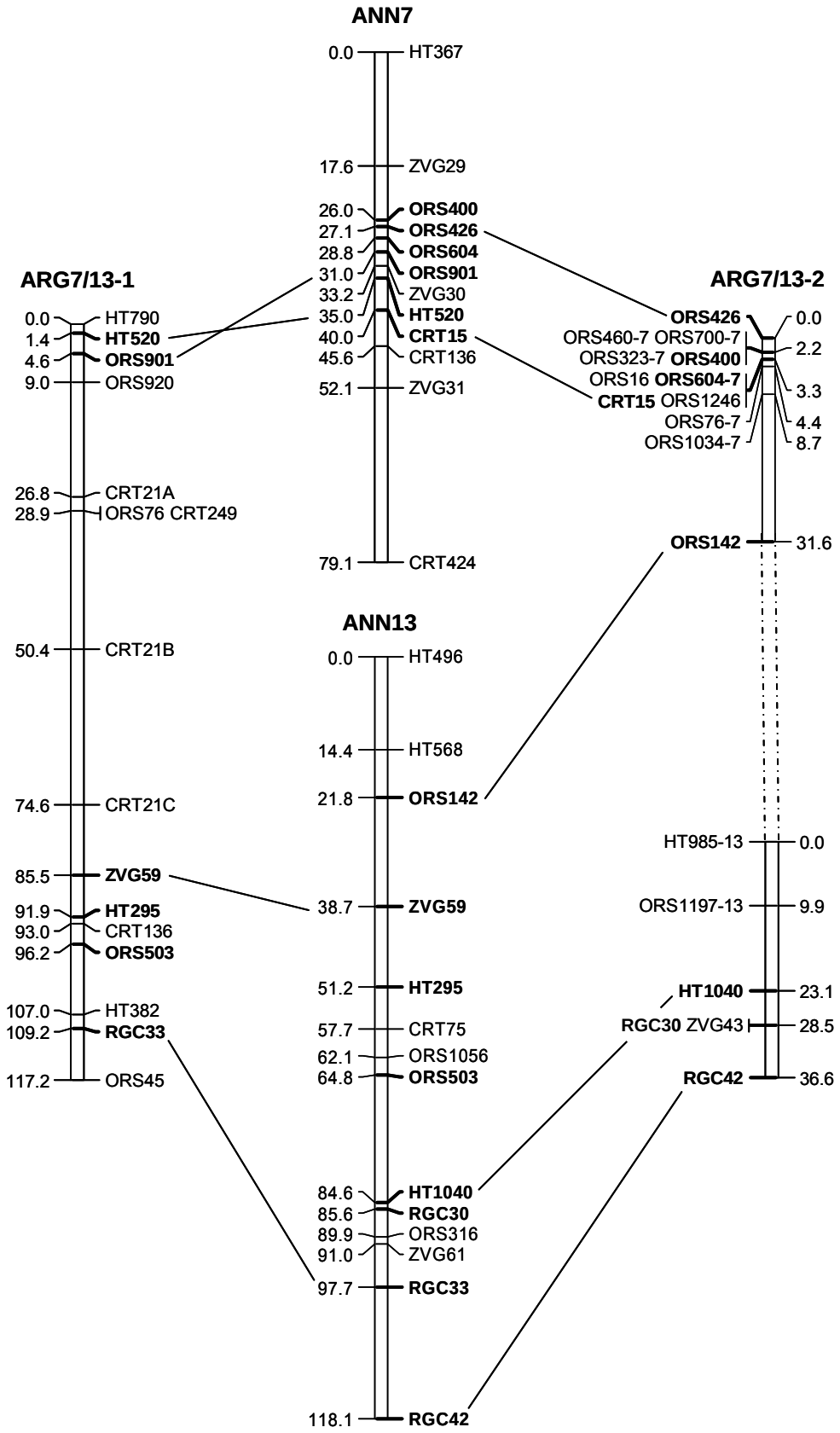
12.3 ORS374
28.3 ORS608
32.3 ORS1219
39.2 ORS1256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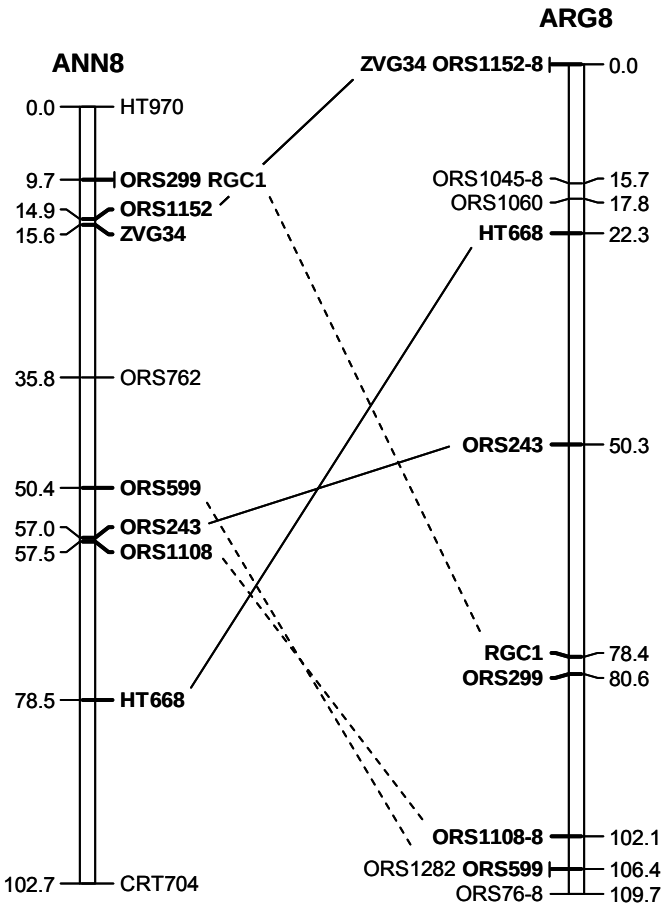
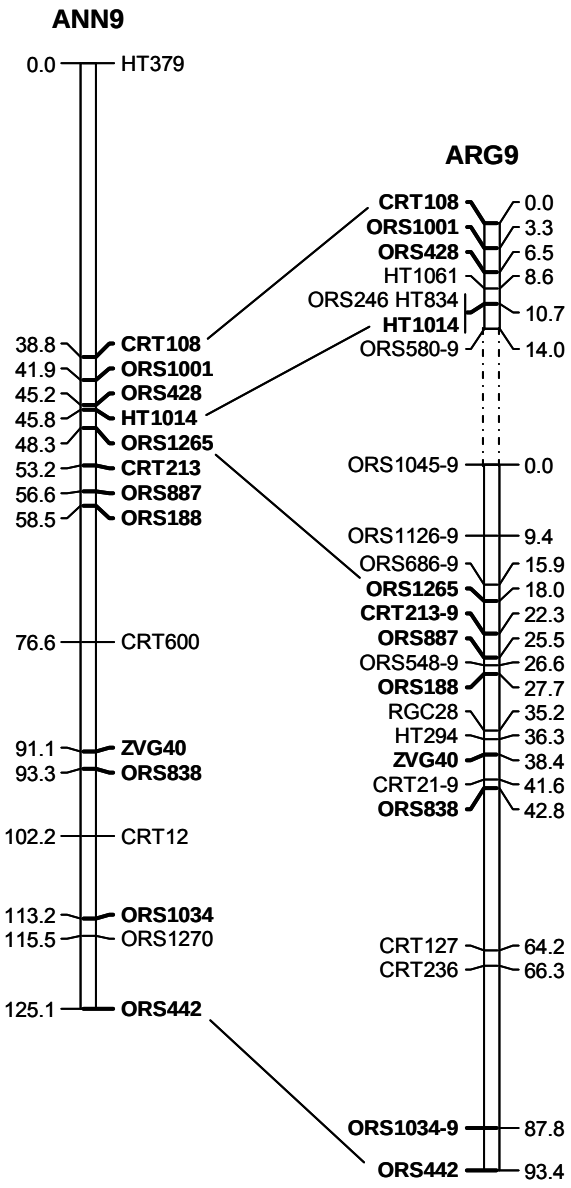
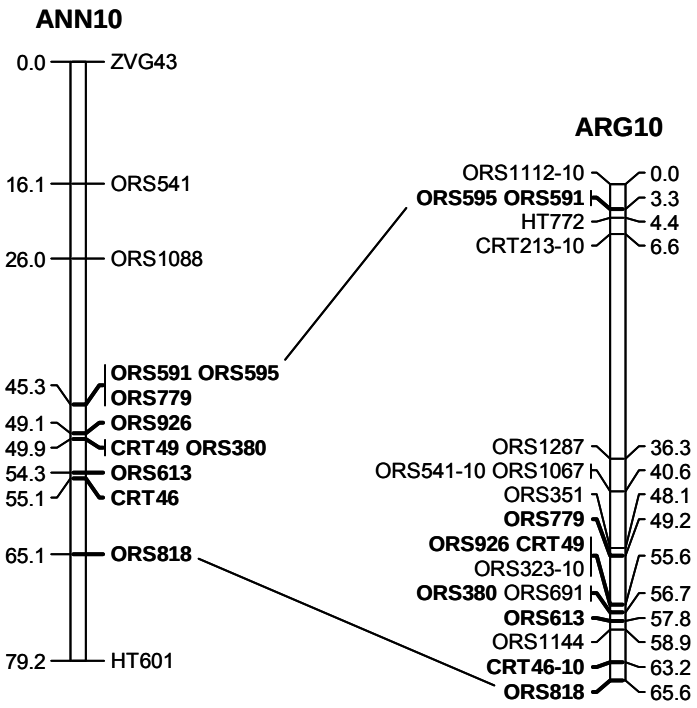
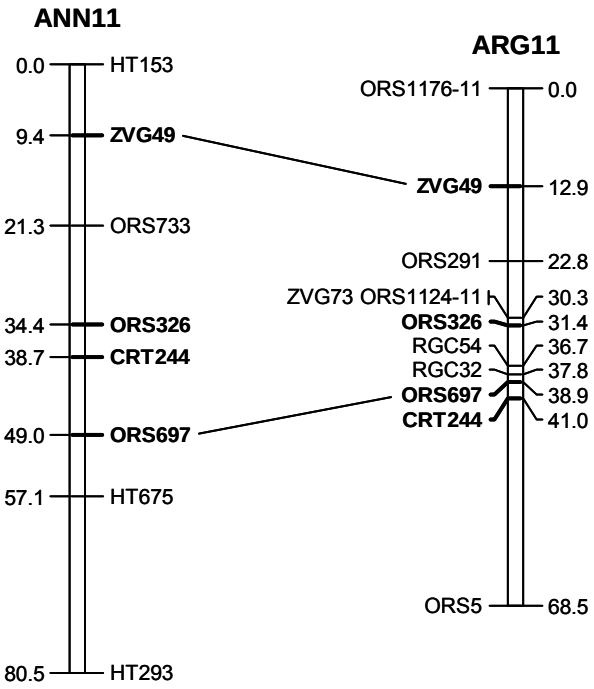
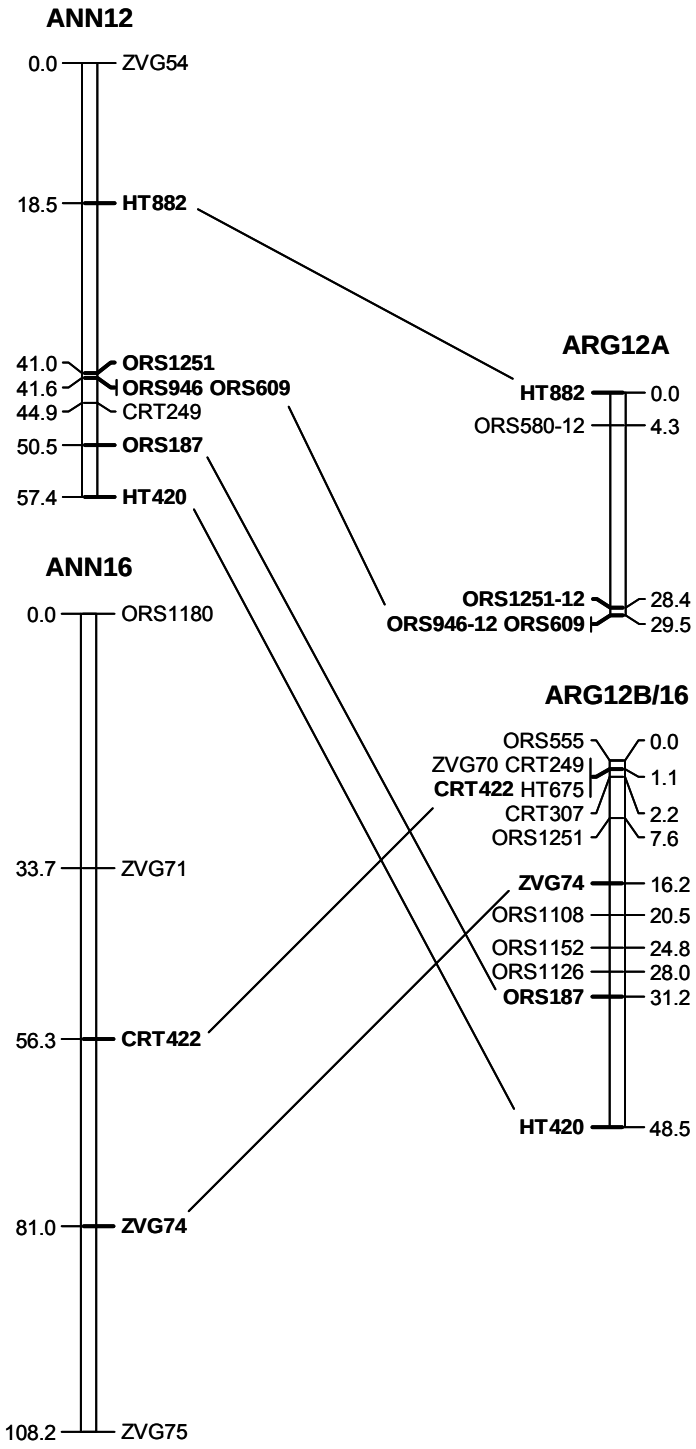
27.1 ORS426
28.8 ORS604
31.0 ORS901
33.2 ZVG30
35.0 HT520
40.0 CRT15
45.6 CRT136
52.1 ZVG31
79.1 CRT424
ARG7/13-1
0.0 HT790
1.4 HT520
4.6 ORS901
9.0 ORS920
26.8 CRT21A
28.9 ORS76 CRT249
50.4 CRT21B
74.6 CRT21C
85.5 ZVG59
91.9 HT295
93.0 CRT136
96.2 ORS503
107.0 HT382
109.2 RGC33
117.2 ORS45
ANN13
0.0 HT496
14.4 HT568
21.8 ORS142
38.7 ZVG59
51.2 HT295
57.7 CRT75
62.1 ORS1056
64.8 ORS503
84.6 HT1040
85.6 RGC30
89.9 ORS316
91.0 ZVG61
97.7 RGC33
118.1 RGC42
ARG7/13-2
ORS426 0.0
ORS460-7 ORS700-7 2.2
ORS323-7 ORS400
ORS16 ORS604-7 3.3
CRT15 ORS1246
ORS76-7 4.4
ORS1034-7 8.7
ORS142 31.6
HT985-13 0.0
ORS1197-13 9.9
HT1040 23.1
RGC30 ZVG43 28.5
RGC42 36.6

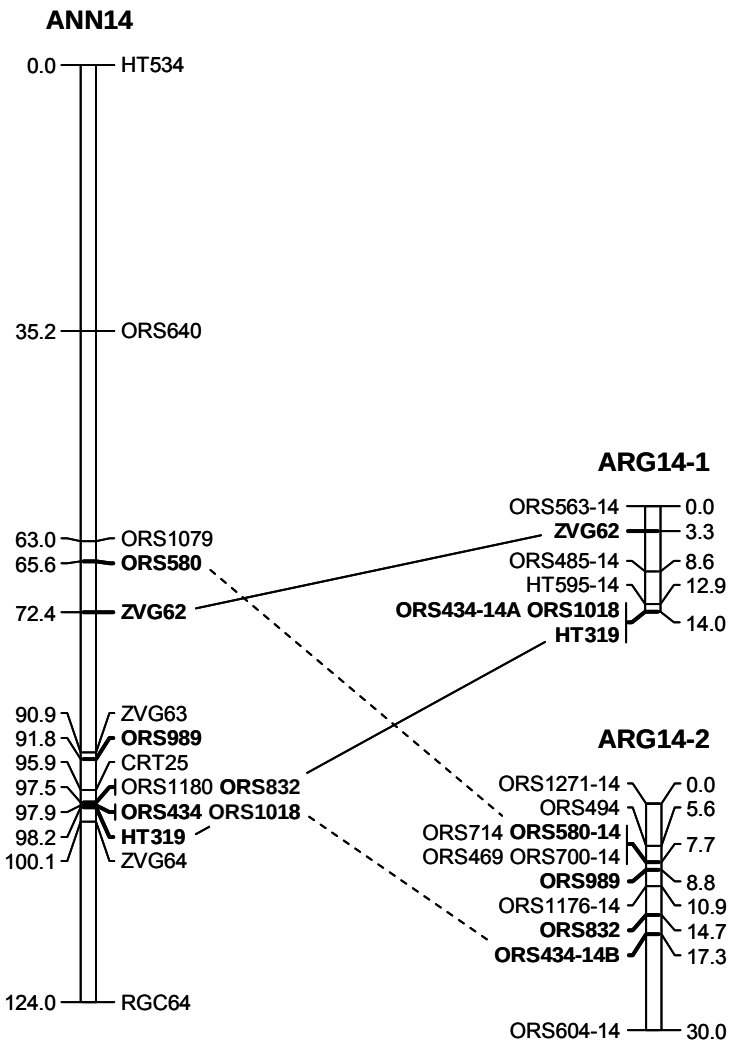

ANN8
ARG8
0.0 HT970
9.7 ORS299 RGC1
14.9 ORS1152
15.6 ZVG34
35.8 ORS762
50.4 ORS599
57.0 ORS243
57.5 ORS1108
78.5 HT668
102.7 CRT704
ZVG34 ORS1152-8 0.0
ORS1045-8 15.7
ORS1060 17.8
HT668 22.3
ORS243 50.3
RGC1 78.4
ORS299 80.6
ORS1108-8 102.1
ORS1282 ORS599 106.4
ORS76-8 109.7


ANN9
0.0 HT379
38.8 CRT108
41.9 ORS1001
45.2 ORS428
45.8 HT1014
48.3 ORS1265
53.2 CRT213
56.6 ORS887
58.5 ORS188
76.6 CRT600
91.1 ZVG40
93.3 ORS838
102.2 CRT12
113.2 ORS1034
115.5 ORS1270
125.1 ORS442
ARG9
CRT108 0.0
ORS1001 3.3
ORS428 6.5
HT1061 8.6
ORS246 HT834 10.7
HT1014
ORS580-9 14.0
ORS1045-9 0.0
ORS1126-9 9.4
ORS686-9 15.9
ORS1265 18.0
CRT213-9 22.3
ORS887 25.5
ORS548-9 26.6
ORS188 27.7
RGC28 35.2
HT294 36.3
ZVG40 38.4
CRT21-9 41.6
ORS838 42.8
CRT127 64.2
CRT236 66.3
ORS1034-9 87.8
ORS442 93.4


ANN10
0.0 ZVG43
16.1 ORS541
26.0 ORS1088
45.3 ORS591 ORS595
ORS779
49.1 ORS926
49.9 CRT49 ORS380
54.3 ORS613
55.1 CRT46
65.1 ORS818
79.2 HT601
ARG10
ORS1112-10 0.0
ORS595 ORS591 3.3
HT772 4.4
CRT213-10 6.6
ORS1287 36.3
ORS541-10 ORS1067 40.6
ORS351 48.1
ORS779 49.2
ORS926 CRT49 55.6
ORS323-10
ORS380 ORS691 56.7
ORS613 57.8
ORS1144 58.9
CRT46-10 63.2
ORS818 65.6


ANN11
0.0 HT153
9.4 ZVG49
21.3 ORS733
34.4 ORS326
38.7 CRT244
49.0 ORS697
57.1 HT675
80.5 HT293
ARG11
ORS1176-11 0.0
ZVG49 12.9
ORS291 22.8
ZVG73 ORS1124-11 30.3
ORS326 31.4
RGC54 36.7
RGC32 37.8
ORS697 38.9
CRT244 41.0
ORS5 68.5


ANN12
0.0 ZVG54
18.5 HT882
41.0 ORS1251
41.6 ORS946 ORS609
44.9 CRT249
50.5 ORS187
57.4 HT420
ANN16
0.0 ORS1180
33.7 ZVG71
56.3 CRT422
81.0 ZVG74
108.2 ZVG75
ARG12A
HT882 0.0
ORS580-12 4.3
ORS1251-12 28.4
ORS946-12 ORS609 29.5
ARG12B/16
ORS555 0.0
ZVG70 CRT249 1.1
CRT422 HT675 2.2
CRT307 7.6
ORS1251
ZVG74 16.2
ORS1108 20.5
ORS1152 24.8
ORS1126 28.0
ORS187 31.2
HT420 48.5


ANN14
0.0 HT534
35.2 ORS640
63.0 ORS1079
65.6 ORS580
72.4 ZVG62
90.9 ZVG63
91.8 ORS989
95.9 CRT25
97.5 ORS1180 ORS832
97.9 ORS434 ORS1018
98.2 HT319
100.1 ZVG64
124.0 RGC64
ARG14-1
ORS563-14 0.0
ZVG62 3.3
ORS485-14 8.6
HT595-14 12.9
ORS434-14A ORS1018 14.0
HT319
ARG14-2
ORS1271-14 0.0
ORS494 5.6
ORS714 ORS580-14 7.7
ORS469 ORS700-14
ORS989 8.8
ORS1176-14 10.9
ORS832 14.7
ORS434-14B 17.3
ORS604-14 30.0

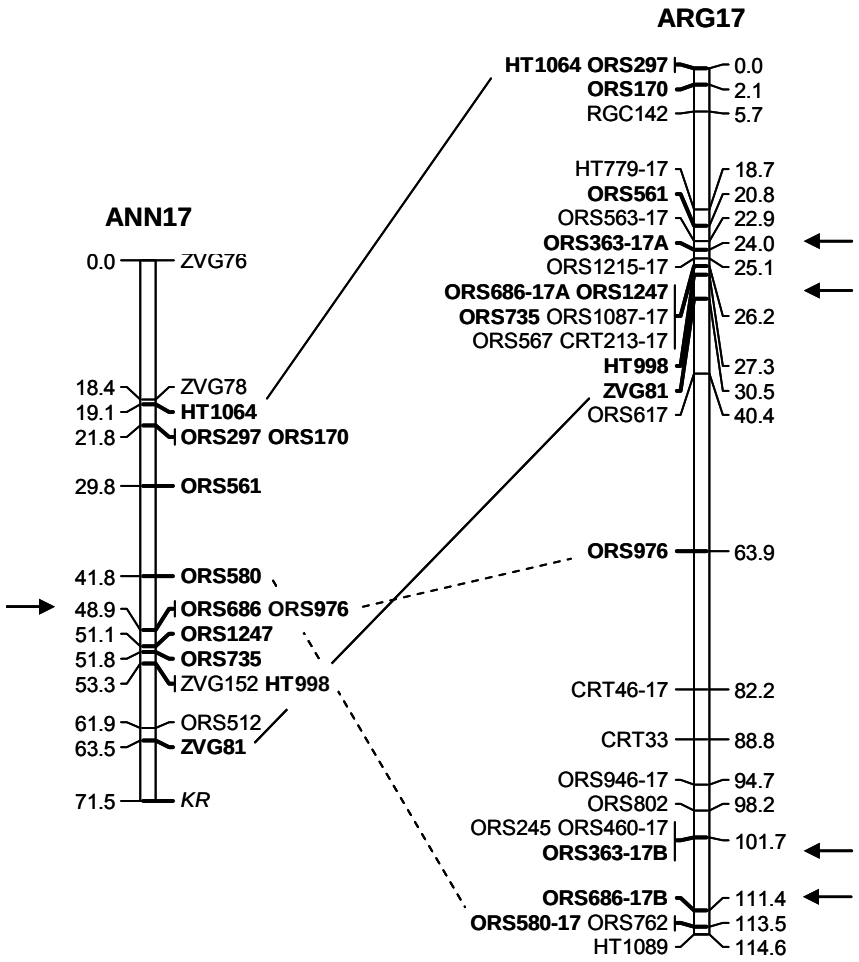

ANN17
0.0 ZVG76
18.4 ZVG78
19.1 HT1064
21.8 ORS297 ORS170
29.8 ORS561
41.8 ORS580
48.9 ORS686 ORS976
51.1 ORS1247
51.8 ORS735
53.3 ZVG152 HT998
61.9 ORS512
63.5 ZVG81
71.5 KR
ARG17
HT1064 ORS297 0.0
ORS170 2.1
RGC142 5.7
HT779-17 18.7
ORS561 20.8
ORS563-17 22.9
ORS363-17A 24.0
ORS1215-17 25.1
ORS686-17A ORS1247
ORS735 ORS1087-17 26.2
ORS567 CRT213-17
HT998 27.3
ZVG81 30.5
ORS617 40.4
ORS976 63.9
CRT46-17 82.2
CRT33 88.8
ORS946-17 94.7
ORS802 98.2
ORS245 ORS460-17
ORS363-17B 101.7
ORS686-17B 111.4
ORS580-17 ORS762 113.5
HT1089 114.6

Figure 2. Translocations, inversions, and duplications inferred by comparative mapping in *H. annuus* (ANN) and *H. argophyllus* (ARG). ARG linkage groups harboring overlapping subsets of independent and duplicated DNA marker loci or independent DNA marker loci only are identified by numerical suffixes (1 and 2). ARG linkage groups harboring inversions are identified by the suffix INV. ARG linkage groups or linkage group segments produced by breakages of ANN linkage groups are identified by letter suffixes (A and B).

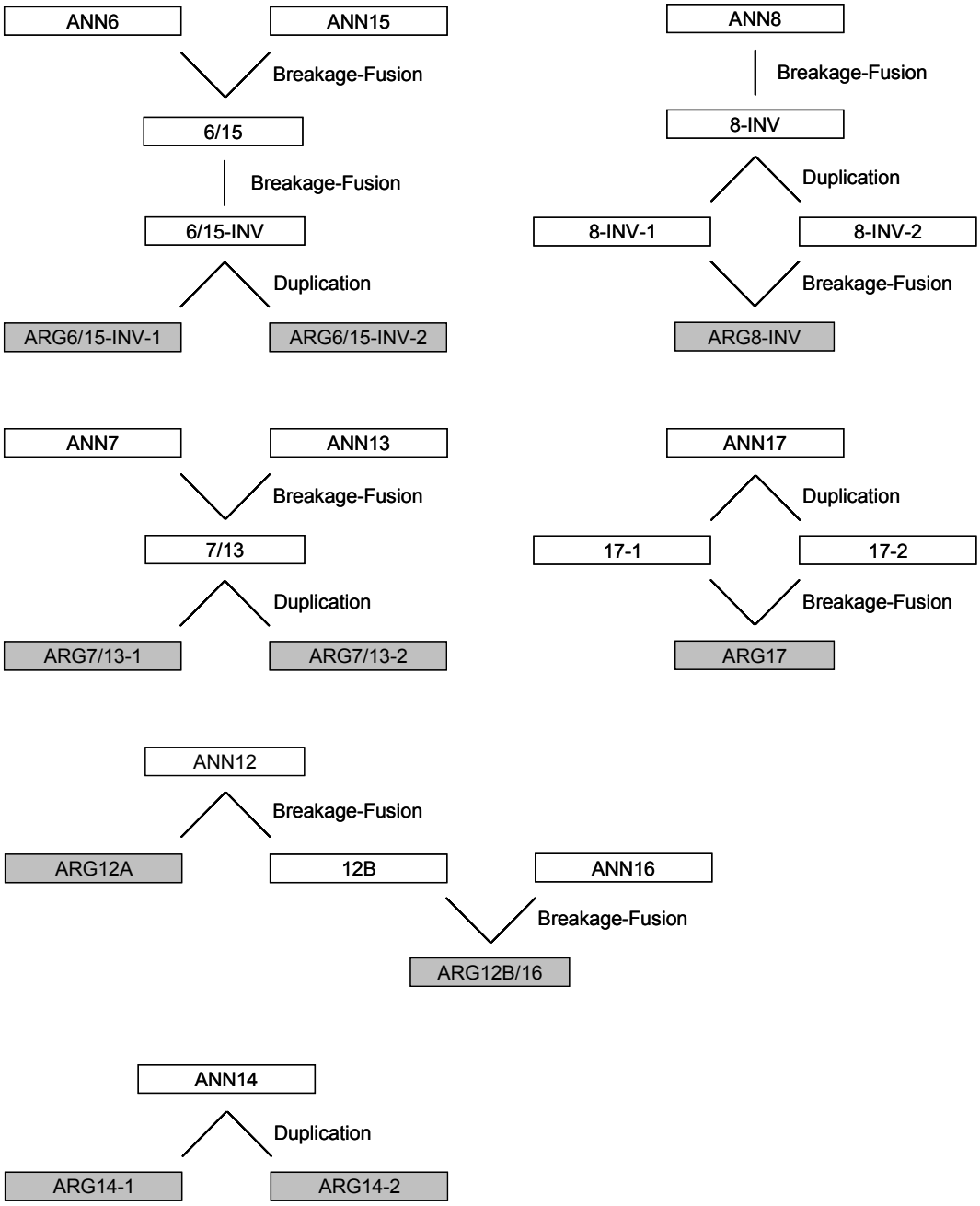
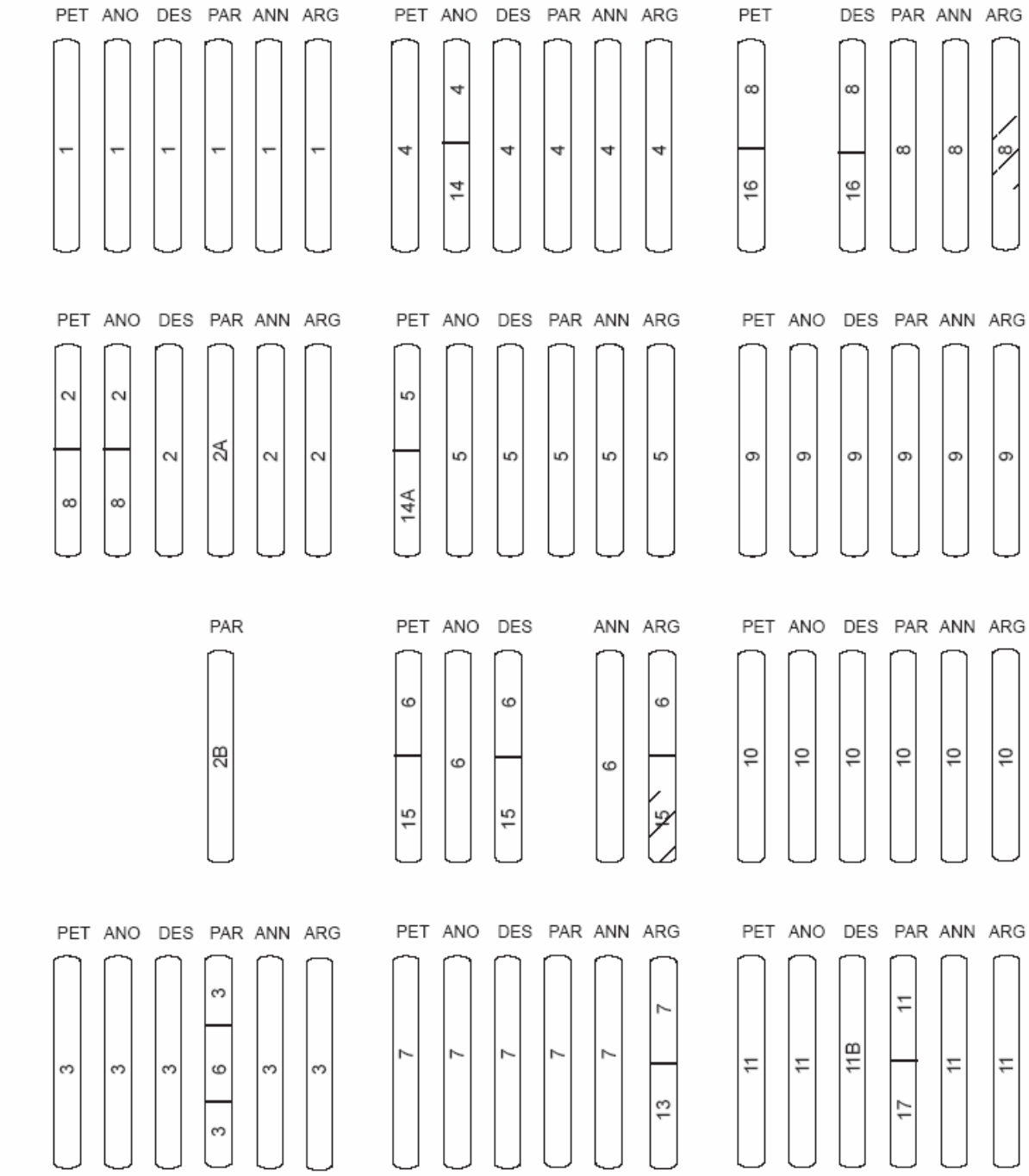

ANN6
ANN15
Breakage-Fusion
6/15
Breakage-Fusion
6/15-INV
Duplication
ARG6/15-INV-1
ARG6/15-INV-2
ANN8
Breakage-Fusion
8-INV
Duplication
8-INV-1
8-INV-2
Breakage-Fusion
ARG8-INV
ANN7
ANN13
Breakage-Fusion
7/13
Duplication
ARG7/13-1
ARG7/13-2
ANN17
Duplication
17-1
17-2
Breakage-Fusion
ARG17
ANN12
Breakage-Fusion
ARG12A
12B
ANN16
Breakage-Fusion
ARG12B/16
ANN14
Duplication
ARG14-1
ARG14-2

Figure 3. Chromosome structures inferred by comparative mapping in *H. annuus* (ANN), *H. anomalus* (ANO), *H. deserticola* (DES), *H. paradoxus* (PAR), *H. petiolaris* (PET), and *H. argophyllus* (ARG). Segments harboring inversions are identified by cross-hatching.


PET ANO DES PAR ANN ARG
1 1 1 1 1 1
PET ANO DES PAR ANN ARG
4 4 14 4 4 4 4
PET DES PAR ANN ARG
8 16 8 16 8 8 8
PET ANO DES PAR ANN ARG
2 8 2 8 2 2A 2 2
PET ANO DES PAR ANN ARG
5 14A 5 5 5 5 5
PET ANO DES PAR ANN ARG
9 9 9 9 9 9
PAR
2B
PET ANO DES ANN ARG
6 15 6 6 15 6 15
PET ANO DES PAR ANN ARG
10 10 10 10 10 10
PET ANO DES PAR ANN ARG
3 3 3 3 6 3 3 3
PET ANO DES PAR ANN ARG
7 7 7 7 7 7 13
PET ANO DES PAR ANN ARG
11 11 11B 11 17 11 11

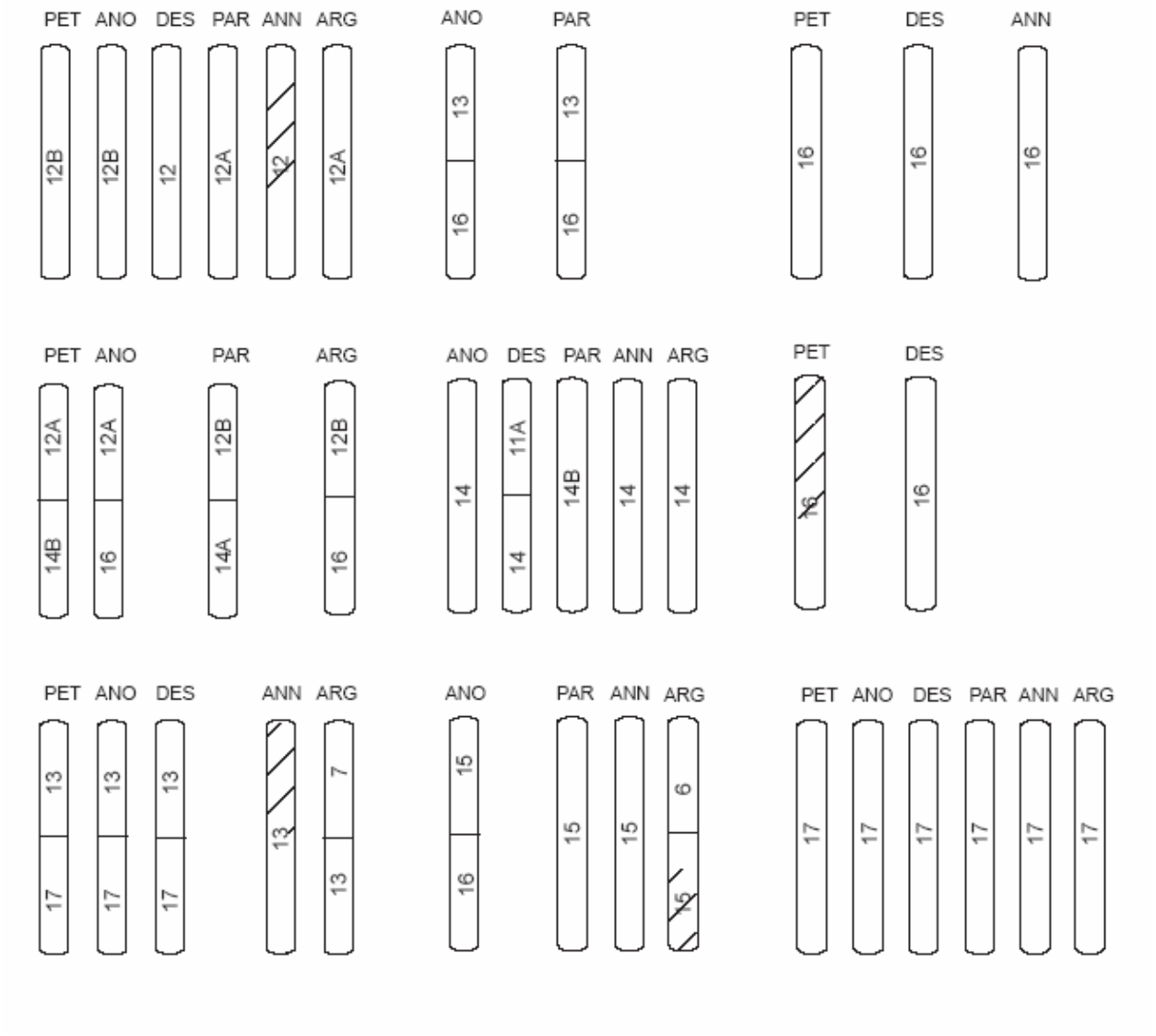

PET ANO DES PAR ANN ARG
12B 12B 12 12A 12 12A
ANO PAR
13 16 13 16
PET DES ANN
16 16 16
PET ANO PAR ARG
12A 14B 12A 16 12B 14A 12B 16
ANO DES PAR ANN ARG
14 11A 14 14B 14 14
PET DES
16 16
PET ANO DES ANN ARG
13 17 13 17 13 17 13 13 7 13
ANO PAR ANN ARG
15 16 15 15 6 5
PET ANO DES PAR ANN ARG
17 17 17 17 17 17

Figure 4. Syntenic chromosomes predicted to be present in ancestors preceeding the divergence of *H. annuus* (ANN), *H. petiolaris* (PET), and *H. argophyllus* (ARG) (proto-PET/ANN/ARG) and *H. annuus* and *H. argophyllus* (proto-ANN/ARG). Numbers identify *H. annuus* linkage groups. Branch points are mean times of divergence and vertical dashed lines flanking branch points are divergence time ranges in million years (adapted from Rieseberg et al. 1991). Chromosomal rearrangements are listed to the right of each branch point.

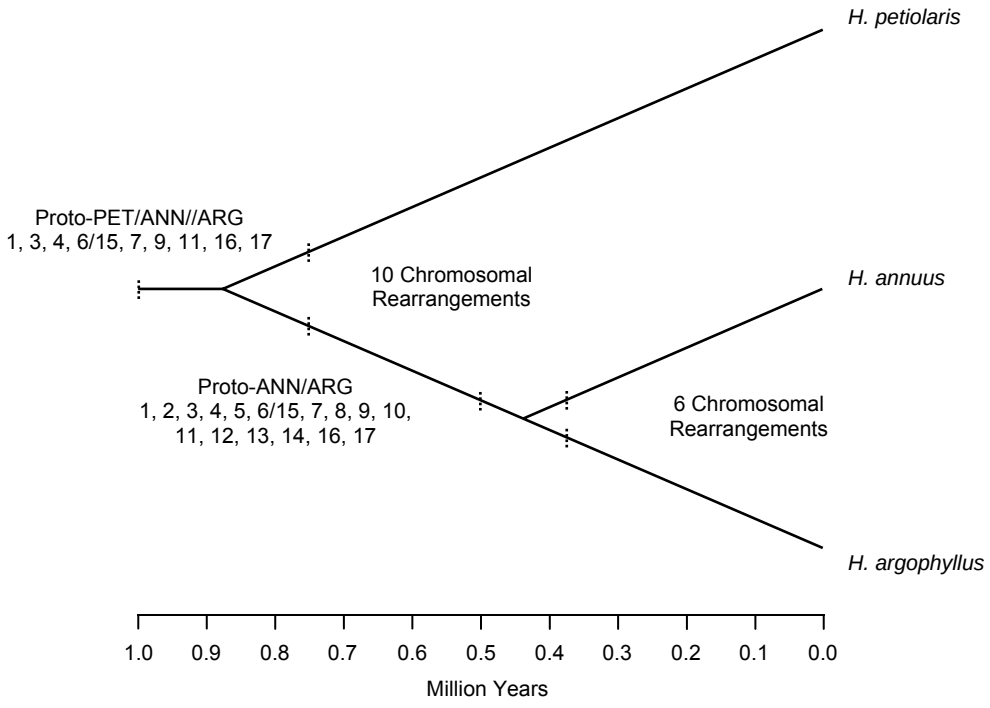
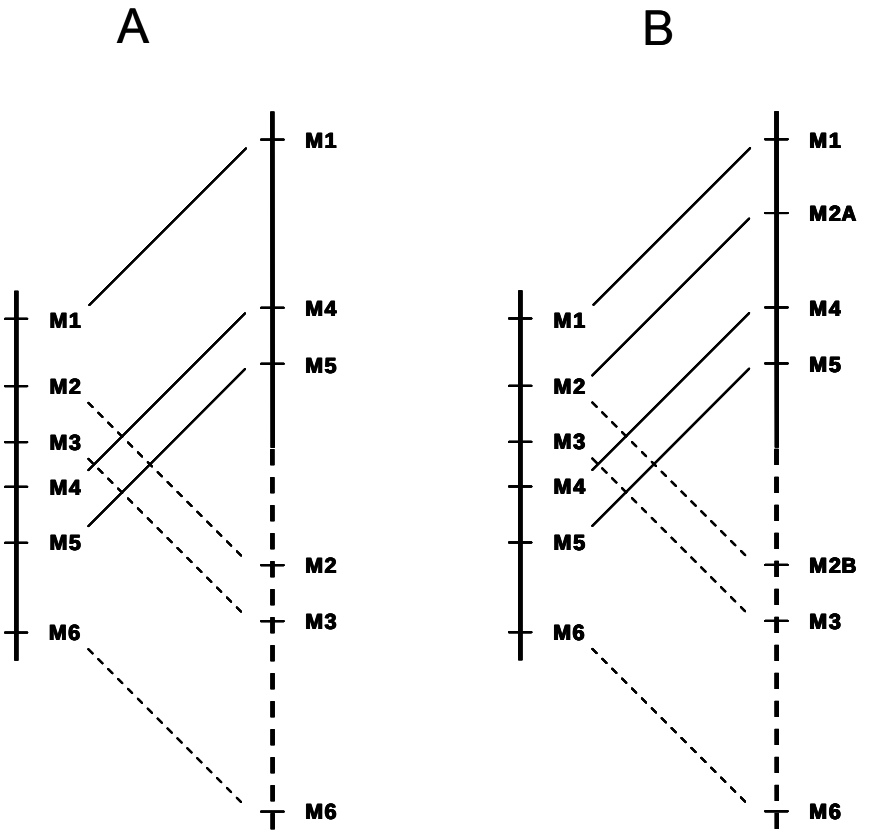


Figure 5. Segmental duplication as revealed by marker overlap. M# represents different marker loci. Dashed lines represent segmental duplication on the right-hand linkage group. A) Overlapping sets of DNA markers in the left linkage group reveal segmental duplication in the right linkage group. B) Overlapping sets of DNA markers, and paralogous duplicated loci (M2A and M2B) reveal segmental duplication in the right linkage group.



CHAPTER 3

CONCLUSIONS

Through mapping of silver-leaf sunflower and comparing it to the public common sunflower map, many unexpected insights were revealed. These insights include: 1) locations of previous introgressions from *H. argophyllus*, 2) a re-evaluation of meiotic differences between *H. annuus* and *H. argophyllus*, 3) a more thorough understanding of the karyotypic evolution of the genus *Helianthus*, and 4) a reassessment of the practical aspects in interspecific breeding programs, especially those concerning *Helianthus* species.

Insights into Previous Introgressions from *H. argophyllus*

Most introgressive breeding using silver-leaf sunflower as a donor parent, has been for traits showing a simple Mendelian inheritance. Resistance phenotypes both abiotic and biotic as well as fertility restoration genes are examples. *H. argophyllus* has provided alleles to the resistance of fungi, moths, and drought as well as genes for fertility restoration (Table 4).

Most notably of the genes transferred from silver-leaf sunflower are those related to resistance to the pathogen *Plasmopara halstedii* (Farl.) Berl.and de Toni (also known as sunflower downy mildew). The first recorded introgression of downy mildew resistance from *ARG* was the gene Pl_8 (Miller and Gulya, 1988; 1991; Tan et al. 1992; Bert et al. 2001). This gene has subsequently been mapped to the bottom of ANN13 in a cluster of resistance genes having CC-NBS-LRR morphology (Bouzidi et al. 2002; Slabaugh et al. 2003; Radwan et al. 2003,2004). In the present study we have shown that the introgressed telomeric segment of ANN13 in cultivated sunflower originally came from an ARG7/13 chromosome.

The other introgression of downy mildew resistance into sunflower was transfer of the gene Pl_{Arg} (Duβle et al., 2004). In the map of the aforementioned publication the gene was mapped to ANN1 and there was a high number of ordering errors, thus the trait was claimed to be telomeric at the top of the linkage group. The researchers saw both suppressed recombination and segregation distortion for this linkage group, and follow-up studies (Leon et al., personal communication) have seen the same. By purposefully ignoring putative escapes (which receive a resistant score) in the phenotypic data they were able to map the gene, and also have colinear order with ANN1 (this study shows that ANN1 and ARG1 are colinear). The study was replicated in two independent populations. The resistance phenotype mapped between the area of suppressed recombination (which includes markers ORS622 and ORS543) and ORS959. It can be stated that as Pl_{Arg} is introgressed from ARG, it also carries the traits of suppressed recombination and segregation distortion as linkage drag.

The final documented case of introgression is of the Rf_3 gene from ARG. Abratti et al. (personal communication) found that the gene for the fertility restoration of PET1 cytoplasm (Rf_1), which has previously been mapped to ANN13 (Kusterer et al. 2005), mapped to ANN7 in a population derived from the inbred line RHA340. RHA340 is a backcross derived introgression line between an *H. argophyllus* wild parent and HA89 as a recurrent parent (Miller and Gulya, 1988; 1991). This locus must have been transferred from an ARG7/13 chromosome to ANN7 in the backcross process.

Insights on Meiotic Abnormalities

Previous cytological studies of F_1 hybrids of common and silver-leaf sunflower have revealed meiotic abnormalities including Anaphase I bridges and multivalent configurations at Diakinesis. In review, the most prevalent meiotic abnormalities (identified at diakinesis), other

than univalents (one to four), were 15 bivalents (II) + 1 tetravalent (IV), 13II + 2IV, 11II + 3IV, and 9II + 4IV (Heiser, 1951; Chandler et al. 1986; Quillet et al. 1995; Manjula et al. 1999; Atalagic et al. 2005). The chromosomes of *H. annuus* and *H. argophyllus* were predicted by Chandler et al. (1986) to carry two reciprocal translocations, a conclusion supported by Quillet et al. (1995).

The expected hybrid PMC cytological configuration based on comparative mapping is predicted to be 11 bivalents (II) and 3 tetravalents (IV). This configuration has previously been reported by Quillet et al. (1995), and Atalagic et al. (2005). Chandler et al. (1986) hypothesized that the only karyotypic differences separating these species were two reciprocal translocations. Comparative mapping of *H. argophyllus* showed no evidence for this hypothesis. Instead what is seen are duplicated chromosomes (the two ARG6/15 and ARG7/13), and a non-reciprocal translocation between linkage groups 12 and 16. The three tetravalents would be formed by associations of various *H. annuus* and *H. argophyllus* chromosomes. One tetravalent is postulated to be formed by associations of chromosomes ANN6, ANN15, ARG6/15-1, and ARG6/15-2. The second is formed by associations of ANN7, ANN13, and ARG7/13-1 and ARG7/13-2. Finally the last tetravalent is expected to be formed by associations between ANN12, ANN16, ARG12A, and ARG12B/16.

Though most studies of *H. argophyllus* cytology didn't detect Anaphase I bridges (Heiser, 1951; Chandler et al. 1986; Quillet et al. 1995) this project showed that inversions were present in the *H. argophyllus* map. Anaphase I bridges were seen in one population by Atalagic et al. (2005). To see if any of these inversions may have had a cytological effect, pollen mother cells (PMCs) of the F_1 hybrids were assessed. Sunflower heads were collected prior to pollen maturity and

Anaphase I cells were observed. Most of the PMCs contained two bridges (Figure 6). The chromosomes identified to have inversions (ARG8 and the ARG6/15 duplicates) are suspected as causing these abnormalities.

Karyotypic Evolution in *Helianthus*

Chromosomal differences found between *H. annuus* and *H. argophyllus* in this study shed additional light on the complicated and rapid nature of karyotypic evolution in the genus *Helianthus* (Table 3). By making three-way comparisons of chromosomal structures in *H. annuus*, *H. petiolaris*, and *H. argophyllus*, a historical reconstruction can be performed to elucidate the origin and persistence of chromosomal rearrangements (Figures 3 and 4). The ancestor of *H. annuus* and *H. argophyllus* (Proto-ANN/ARG; Figure 4) had a minimum of 16 chromosomes and most likely resembled the karyotype of *H. annuus*, with the exception of having a fused 6/15 chromosome. From comparisons with this ancestor, many changes can be placed on either the ANN or the ARG lineage. Only one event, the fission of an ancestral 6/15 chromosome to form ANN6 and 15 could be attributed to the branch leading to *H. annuus*. All other karyotypic differences between ANN and ARG seem to have arisen in the ARG lineage. Two of the events are a fusion of the ancestral chromosomes 7 and 13 as well as their subsequent duplication. A second pair are the inversion event on the ancestral 6/15 chromosome and its subsequent duplication. The last pair of events that could be placed on the ARG lineage was the breakage of the ancestral chromosome 12 and its subsequent fusion to ancestral chromosome 16. The putative segmental duplications of ancestral chromosome 8 and 17 could not be placed with certainty on the ARG lineage even though the presence of duplicated inversion on ARG8 suggests that the fusion of duplicated ancestral 8 chromosomes occurred on this lineage.

Chromosomal and Segmental Duplications in *Helianthus*

Most eukaryotic genomes are of polyploid origin (Masterson, 1994; Spring, 1997; Wolfe and Shields, 1997; Matzke et al. 1999; Comai, 2000; Wendel, 2000). Sunflower is an ancient allopolyploid (paleopolyploid) with a physically large genome (3,000-3,400 Mb) and high frequency of duplicated loci (Berry et al. 1996; Gentzbittel et al. 1999; Tang et al. 2002, 2003; Baack et al. 2005). The presence of duplicated loci has not shed light on this ancient allopolyploid event, though several recent chromosomal or segmental duplications were identified between *H. annuus* and *H. argophyllus* in the present study (Figure 1). Multiple studies have shown that duplicated genes have one of four fates: subfunctionalization, neofunctionalization, silencing, or loss (Matzke et al. 1999; Comai, 2000; Wendel, 2000; Liu et al. 2001; Shaked et al. 2001; Han et al. 2003; Langham et al. 2004; Pires et al. 2004a,b; Kovarik et al. 2005; Lukens et al. 2006). Several of the architectural differences found in *H. annuus* and *H. argophyllus* chromosomes seem to have been produced by fusing duplicated chromosomes or chromosome segments (ARG8, ARG14, and ARG17) or by duplicating chromosomes (ARG7/13-1 and 7/13-2 and ARG6/15-1 and 6/15-2) (Figure 1). It is unknown how much divergence has occurred for the genes on duplicated segments, though it is expected that there will be differences in gene use due to genetic or epigenetic modification.

Using Silver-leaf Sunflower in Breeding

The comparative mapping analyses described here create genomic blueprints for designing breeding programs for efficiently transferring *H. argophyllus* alleles into *H. annuus* through marker-assisted selection, and for broadening the utility of *H. argophyllus* as a source of genetic diversity for enhancing drought tolerance, seed yield, and other complex traits in common sunflower. Because the merits of wild species as donors of favorable alleles for enhancing

complex traits usually cannot be ascertained from the phenotypes of the wild species donors *per se*, advanced backcross QTL discovery and segmental IL development strategies should have significant merit for identifying and transferring favorable alleles for complex traits in sunflower (Eshed and Zamir, 1995,1996; Tanksley and Nelson, 1996; Zamir, 2001; Morgante and Salamini, 2003; Gur and Zamir, 2004).

The number of chromosomal rearrangements and duplications found in *H. argophyllus* create complications for IL development which are not found in species where donor and recurrent parent genomes are nearly or completely colinear. Even though this is the case, several *H. annuus* and *H. argophyllus* chromosomes are colinear (1, 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 (8, 14, and 17) (Figure 1 and 3).

The presence of duplications in *H. argophyllus* raises several questions and could have important consequences for introgression line (IL) development. Can complete IL catalogs be developed for common sunflower using silver-leaf sunflower as the donor? When developing ILs for loci carried on duplicated *H. argophyllus* chromosomes or chromosomal segments, do the duplicated chromosomes or chromosomal segments pair with equal probability with the homologous ANN segment in interspecific hybrids? Do the ends of chromosomes produced by end-to-end fusions of duplications (ARG8, 14, and 17) pair with the ends of homologous ANN chromosomes (ANN8, 14, and 17), or is pairing offset? Do duplicated chromosomes produced by end-to-end fusions of two ANN chromosomes (ARG6/15-1 and -2 and ARG7/13-1 and -2) pair with the individual ANN chromosomes present in the fused ARG chromosomes, e.g., do ANN7 and ANN13 pair with ARG7/13-1 and ARG7/13-2 with equal probability in interspecific hybrids? Can two *H. annuus* ILs be developed for each duplicated segment found in *H. argophyllus*? If loci

in duplicated *H. argophyllus* segments have undergone genetic and epigenetic changes since duplication will *H. annuus* ILs carrying different duplicated *H. argophyllus* segments have asymmetrical gene compositions? Several of these questions can perhaps be answered by identifying and graphical genotyping the spectrum of recombinants produced among backcross progeny, and by developing wild ILs using *H. argophyllus* as the donor.

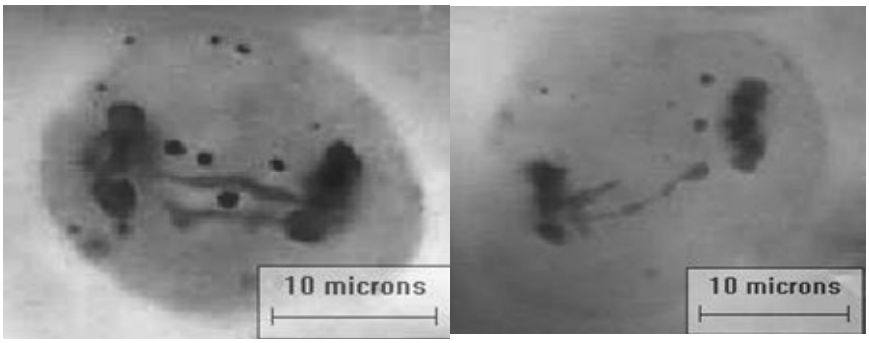
Final Remark

Comparative mapping in common and silver-leaf sunflower has shown that the rapid rate of karyotypic evolution seen in all *Helianthus* species thus far is real, and has shown that aneuploidy (duplication of chromosomes) can be fixed in diploid species. This study has also raised questions about segmental and chromosomal duplications in other unmapped species and questions the validity of using cytological inferences alone in planning breeding programs. Many questions about the effects of duplications on breeding are still to be tackled but having the map of *H. argophyllus* is a valuable tool for starting to address them.

Table 4. Introgressions from Silver-leaf Sunflower.

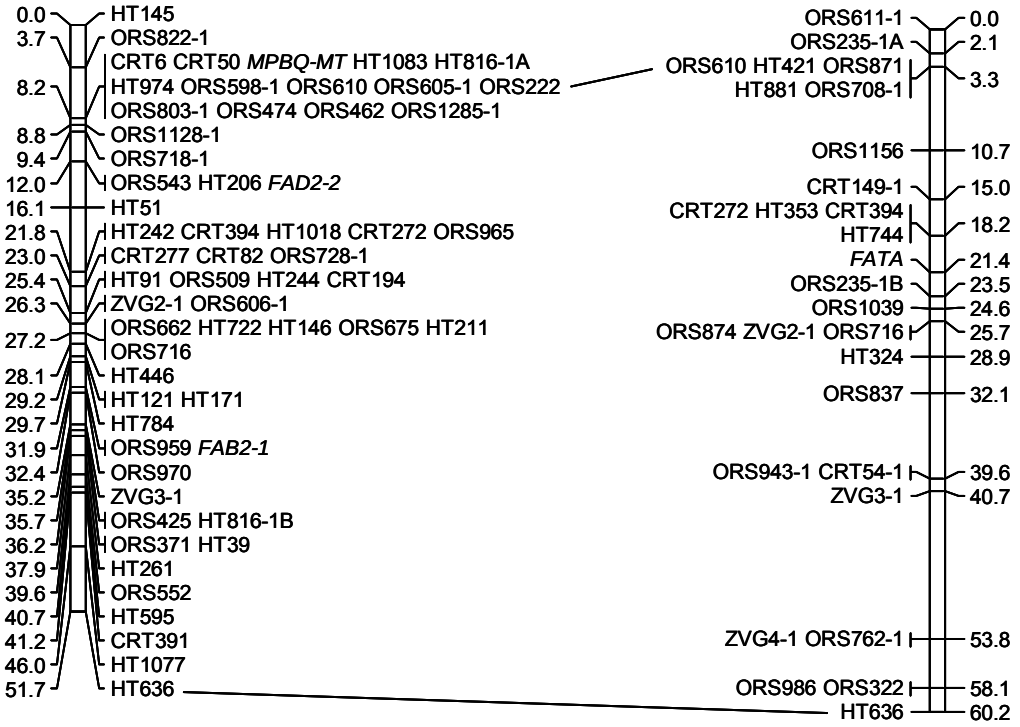
Trait	Target	Gene or Product	ARG source	Introgression Line	ANN LG	Citation
Black rust resistance (Race 4)	*Puccinia helianthi* Schw.	R_{ADV}	ARG415 (Texas)	RHA340	13	Miller and Gulya (1988), Quresh et al. (1993), Unpublished Data
Downy mildew resistance	*Plasmopara halstedii* (Farl.) Berl. & de Toni	Pl_8	ARG415 (Texas)	RHA340, QIR8	13	Miller and Gulya (1991), Seiler (1991a,b), Gulya (2000), Bert et al. (2001), Slabaugh et al. (2003), Radwan et al. (2004)
Downy mildew resistance	*Plasmopara halstedii* (Farl.) Berl. & de Toni	Pl_{Arg}	ARG1575 (Florida)	Arg1575-2, RHA419, RHA420	1	Duβle et al. (2004), Unpublished Data
Downy mildew resistance	*Plasmopara halstedii* (Farl.) Berl. & de Toni	Pl_{ADV}	Unknown	29004	1	Unpublished Data
Sclerotinia resistance	*Sclerotinia sclerotiorum* (Lib.) de Bary	Unknown	ARG1575 (Florida)	BE94-186-01, -02, ARG-283, ARG1575-1,-3	NM	Degener et al. (1999)
Phomopsis resistance	*Diaporthe helianthi* Munt-Cvet et al.	3 interacting loci	MPHE92 (Morocco)	NSH45, LR2, LR4-17	NM	Besnard et al. (1997), Viguié et al. (2000), Langar et al. (2002)
Sunflower moth resistance	*Homoeosoma electellum* (Hulst)	Argophyllin-A and B, Eupatolide, 16-α-hydroxy-kaurane	NA	NA	NM	Rogers et al. (1987)
PET1 cytoplasm fertility restoration	NA	Rf_3	ARG415 (Texas)	RHA340	7	Miller and Gulya (1988), Unpublished Data
Osmotic adjustment	NA	DRS26	NA	T^-	NM	Serieys et al. (1988), Jamaux et al. (1997)

Figure 6. Pollen Mother Cells of F1 Hybrids Showing Two Anaphase I Bridges.

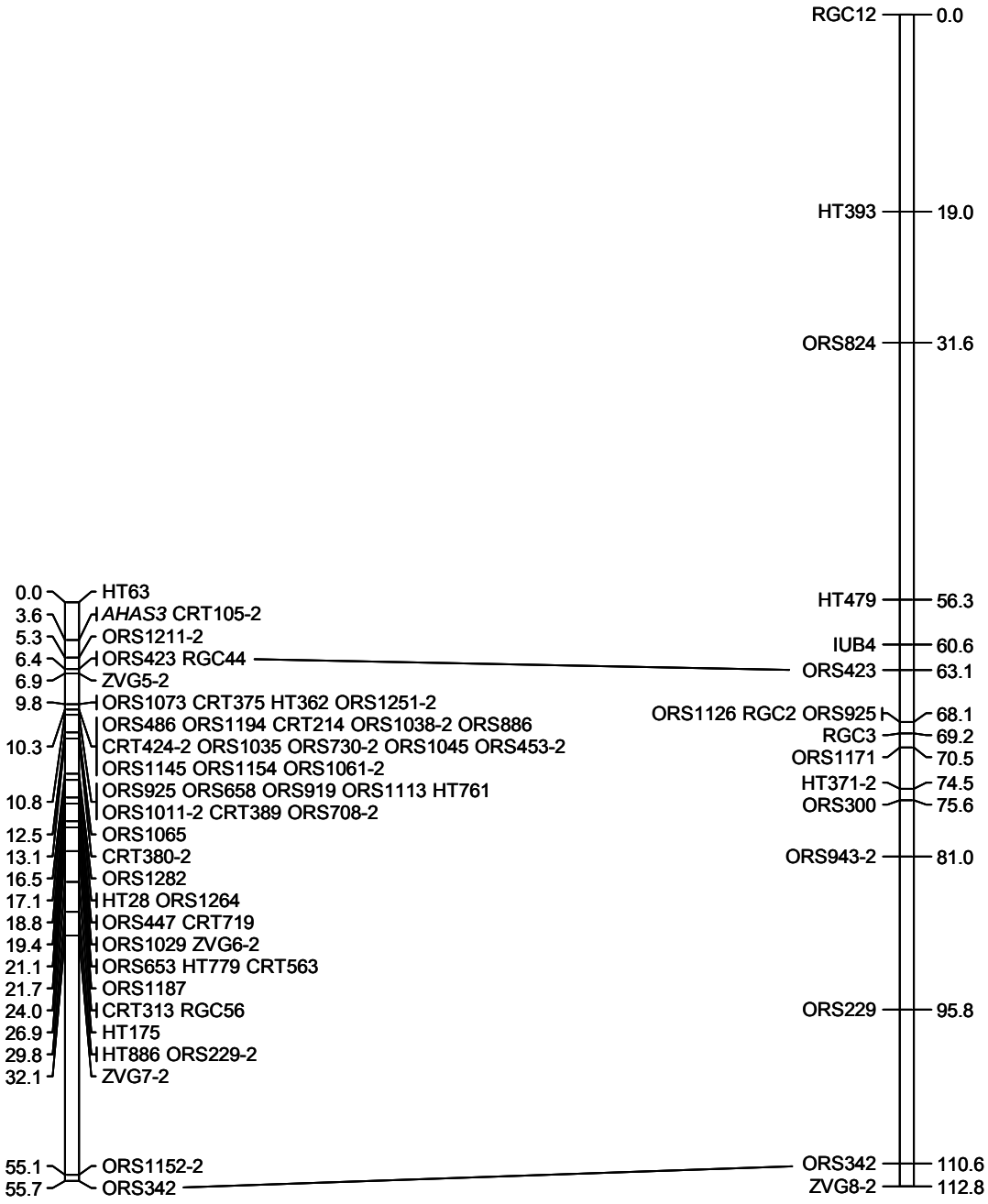


Supplemental Figure 1. Comparative alignments of *H. annuus* linkage groups produced by mapping 1,531 SSR, INDEL, SNP, and SSCP markers in the RHA280 x RHA801 recombinant inbred line (RIL) and NMS373 x (NMS373 x ANN1811) BC_1 mapping populations (left and right hand linkage groups in each pair, respectively). ORS and CRT prefixes identify non-genic SSR markers, the ZVG prefix identifies genic-INDEL or SNP markers, the HT prefix identifies genic-SSR or INDEL markers, and the RGC prefix identifies nucleotide binding site leucine rich repeat (NBS-LRR) SSCP markers.

ANN1


0.0 HT145
3.7 ORS822-1
CRT6 CRT50 *MPBQ-MT* HT1083 HT816-1A
8.2 HT974 ORS598-1 ORS610 ORS605-1 ORS222
ORS803-1 ORS474 ORS462 ORS1285-1
8.8 ORS1128-1
9.4 ORS718-1
12.0 ORS543 HT206 *FAD2-2*
16.1 HT51
21.8 HT242 CRT394 HT1018 CRT272 ORS965
23.0 CRT277 CRT82 ORS728-1
25.4 HT91 ORS509 HT244 CRT194
26.3 ZVG2-1 ORS606-1
27.2 ORS662 HT722 HT146 ORS675 HT211
ORS716
28.1 HT446
29.2 HT121 HT171
29.7 HT784
31.9 ORS959 *FAB2-1*
32.4 ORS970
35.2 ZVG3-1
35.7 ORS425 HT816-1B
36.2 ORS371 HT39
37.9 HT261
39.6 ORS552
40.7 HT595
41.2 CRT391
46.0 HT1077
51.7 HT636
ORS611-1 0.0
ORS235-1A 2.1
ORS610 HT421 ORS871
HT881 ORS708-1 3.3
ORS1156 10.7
CRT149-1 15.0
CRT272 HT353 CRT394
HT744 18.2
FATA 21.4
ORS235-1B 23.5
ORS1039 24.6
ORS874 ZVG2-1 ORS716 25.7
HT324 28.9
ORS837 32.1
ORS943-1 CRT54-1 39.6
ZVG3-1 40.7
ZVG4-1 ORS762-1 53.8
ORS986 ORS322 58.1
HT636 60.2

ANN2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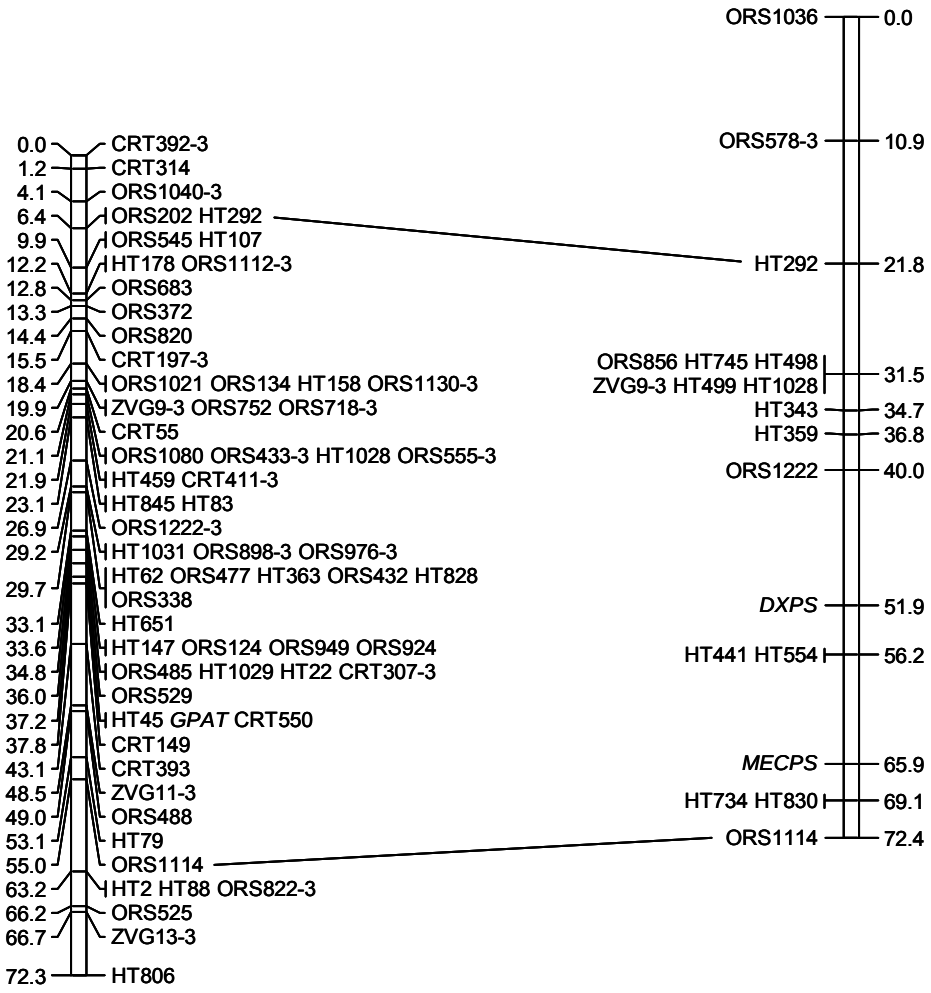
HT393 19.0
ORS824 31.6
0.0 HT63
3.6 AHAS3 CRT105-2
5.3 ORS1211-2
6.4 ORS423 RGC44
6.9 ZVG5-2
9.8 ORS1073 CRT375 HT362 ORS1251-2
ORS486 ORS1194 CRT214 ORS1038-2 ORS886
10.3 CRT424-2 ORS1035 ORS730-2 ORS1045 ORS453-2
ORS1145 ORS1154 ORS1061-2
ORS925 ORS658 ORS919 ORS1113 HT761
10.8 ORS1011-2 CRT389 ORS708-2
12.5 ORS1065
13.1 CRT380-2
16.5 ORS1282
17.1 HT28 ORS1264
18.8 ORS447 CRT719
19.4 ORS1029 ZVG6-2
21.1 ORS653 HT779 CRT563
21.7 ORS1187
24.0 CRT313 RGC56
26.9 HT175
29.8 HT886 ORS229-2
32.1 ZVG7-2
HT479 56.3
IUB4 60.6
ORS423 63.1
ORS1126 RGC2 ORS925 68.1
RGC3 69.2
ORS1171 70.5
HT371-2 74.5
ORS300 75.6
ORS943-2 81.0
ORS229 95.8
55.1 ORS1152-2
55.7 ORS342
ORS342 110.6
ZVG8-2 112.8

ANN3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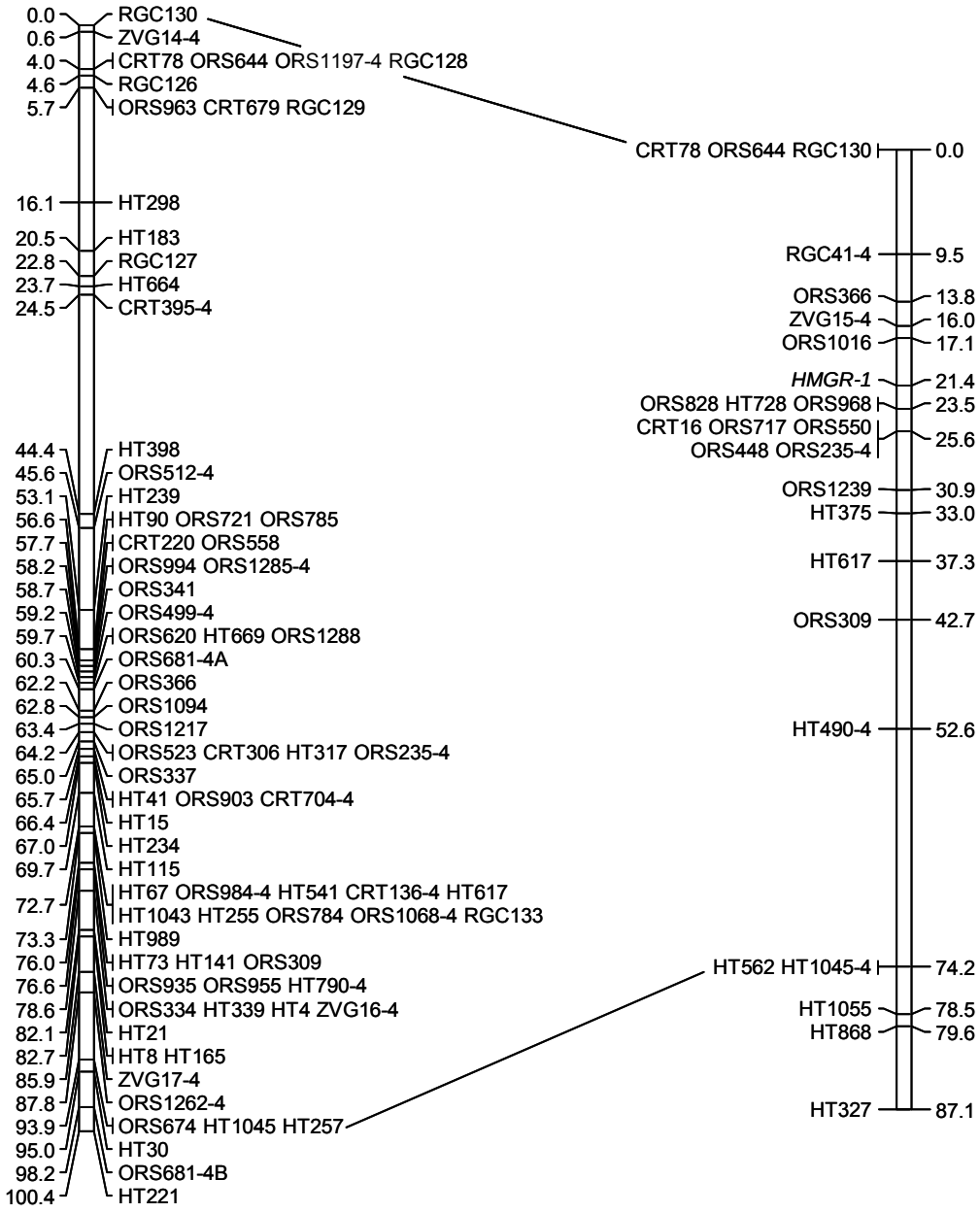
4.1 ORS1040-3
6.4 ORS202 HT292
9.9 ORS545 HT107
12.2 HT178 ORS1112-3
12.8 ORS683
13.3 ORS372
14.4 ORS820
15.5 CRT197-3
18.4 ORS1021 ORS134 HT158 ORS1130-3
19.9 ZVG9-3 ORS752 ORS718-3
20.6 CRT55
21.1 ORS1080 ORS433-3 HT1028 ORS555-3
21.9 HT459 CRT411-3
23.1 HT845 HT83
26.9 ORS1222-3
29.2 HT1031 ORS898-3 ORS976-3
29.7 HT62 ORS477 HT363 ORS432 HT828
ORS338
33.1 HT651
33.6 HT147 ORS124 ORS949 ORS924
34.8 ORS485 HT1029 HT22 CRT307-3
36.0 ORS529
37.2 HT45 GPAT CRT550
37.8 CRT149
43.1 CRT393
48.5 ZVG11-3
49.0 ORS488
53.1 HT79
55.0 ORS1114
63.2 HT2 HT88 ORS822-3
66.2 ORS525
66.7 ZVG13-3
72.3 HT806
ORS1036 0.0
ORS578-3 10.9
HT292 21.8
ORS856 HT745 HT498 31.5
ZVG9-3 HT499 HT1028
HT343 34.7
HT359 36.8
ORS1222 40.0
DXPS 51.9
HT441 HT554 56.2
MECPS 65.9
HT734 HT830 69.1
ORS1114 72.4

ANN4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0.6 ZVG14-4
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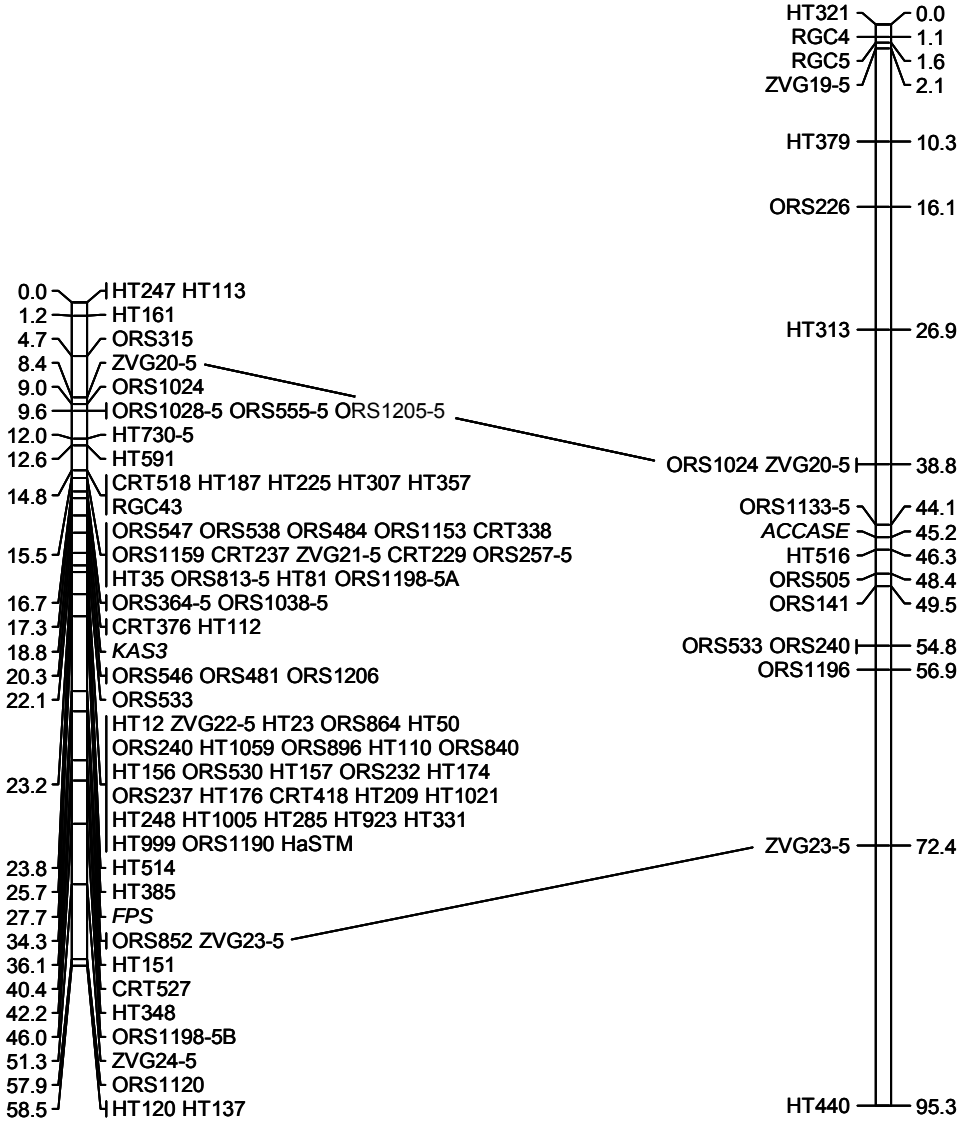
4.6 RGC126
5.7 ORS963 CRT679 RGC129
16.1 HT298
20.5 HT183
22.8 RGC127
23.7 HT664
24.5 CRT395-4
44.4 HT398
45.6 ORS512-4
53.1 HT239
56.6 HT90 ORS721 ORS785
57.7 CRT220 ORS558
58.2 ORS994 ORS1285-4
58.7 ORS341
59.2 ORS499-4
59.7 ORS620 HT669 ORS1288
60.3 ORS681-4A
62.2 ORS366
62.8 ORS1094
63.4 ORS1217
64.2 ORS523 CRT306 HT317 ORS235-4
65.0 ORS337
65.7 HT41 ORS903 CRT704-4
66.4 HT15
67.0 HT234
69.7 HT115
72.7 HT67 ORS984-4 HT541 CRT136-4 HT617
HT1043 HT255 ORS784 ORS1068-4 RGC133
73.3 HT989
76.0 HT73 HT141 ORS309
76.6 ORS935 ORS955 HT790-4
78.6 ORS334 HT339 HT4 ZVG16-4
82.1 HT21
82.7 HT8 HT165
85.9 ZVG17-4
87.8 ORS1262-4
93.9 ORS674 HT1045 HT257
95.0 HT30
98.2 ORS681-4B
100.4 HT221
CRT78 ORS644 RGC130 0.0
RGC41-4 9.5
ORS366 13.8
ZVG15-4 16.0
ORS1016 17.1
HMGR-1 21.4
ORS828 HT728 ORS968 23.5
CRT16 ORS717 ORS550 25.6
ORS448 ORS235-4
ORS1239 30.9
HT375 33.0
HT617 37.3
ORS309 42.7
HT490-4 52.6
HT562 HT1045-4 74.2
HT1055 78.5
HT868 79.6
HT327 87.1

ANN5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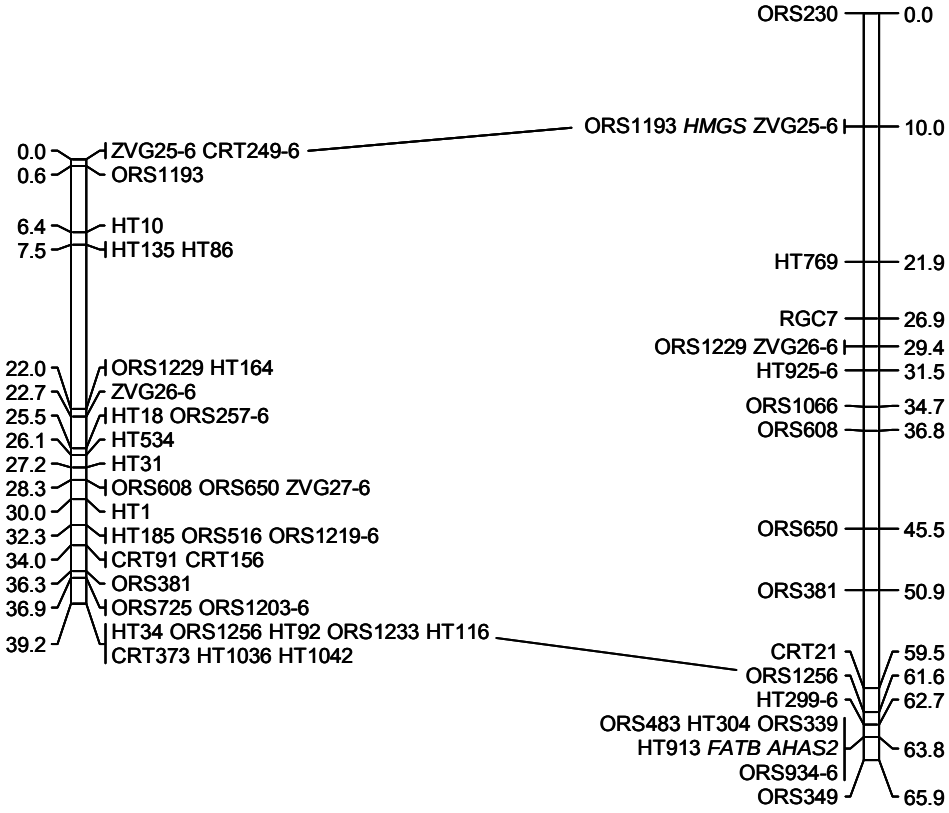
8.4 ZVG20-5
9.0 ORS1024
9.6 ORS1028-5 ORS555-5 ORS1205-5
12.0 HT730-5
12.6 HT591
14.8 CRT518 HT187 HT225 HT307 HT357
RGC43
ORS547 ORS538 ORS484 ORS1153 CRT338
15.5 ORS1159 CRT237 ZVG21-5 CRT229 ORS257-5
HT35 ORS813-5 HT81 ORS1198-5A
16.7 ORS364-5 ORS1038-5
17.3 CRT376 HT112
18.8 KAS3
20.3 ORS546 ORS481 ORS1206
22.1 ORS533
HT12 ZVG22-5 HT23 ORS864 HT50
ORS240 HT1059 ORS896 HT110 ORS840
HT156 ORS530 HT157 ORS232 HT174
23.2 ORS237 HT176 CRT418 HT209 HT1021
HT248 HT1005 HT285 HT923 HT331
HT999 ORS1190 HaSTM
23.8 HT514
25.7 HT385
27.7 FPS
34.3 ORS852 ZVG23-5
36.1 HT151
40.4 CRT527
42.2 HT348
46.0 ORS1198-5B
51.3 ZVG24-5
57.9 ORS1120
58.5 HT120 HT137
HT321 0.0
RGC4 1.1
RGC5 1.6
ZVG19-5 2.1
HT379 10.3
ORS226 16.1
HT313 26.9
ORS1024 ZVG20-5 38.8
ORS1133-5 44.1
ACCASE 45.2
HT516 46.3
ORS505 48.4
ORS141 49.5
ORS533 ORS240 54.8
ORS1196 56.9
ZVG23-5 72.4
HT440 95.3

ANN6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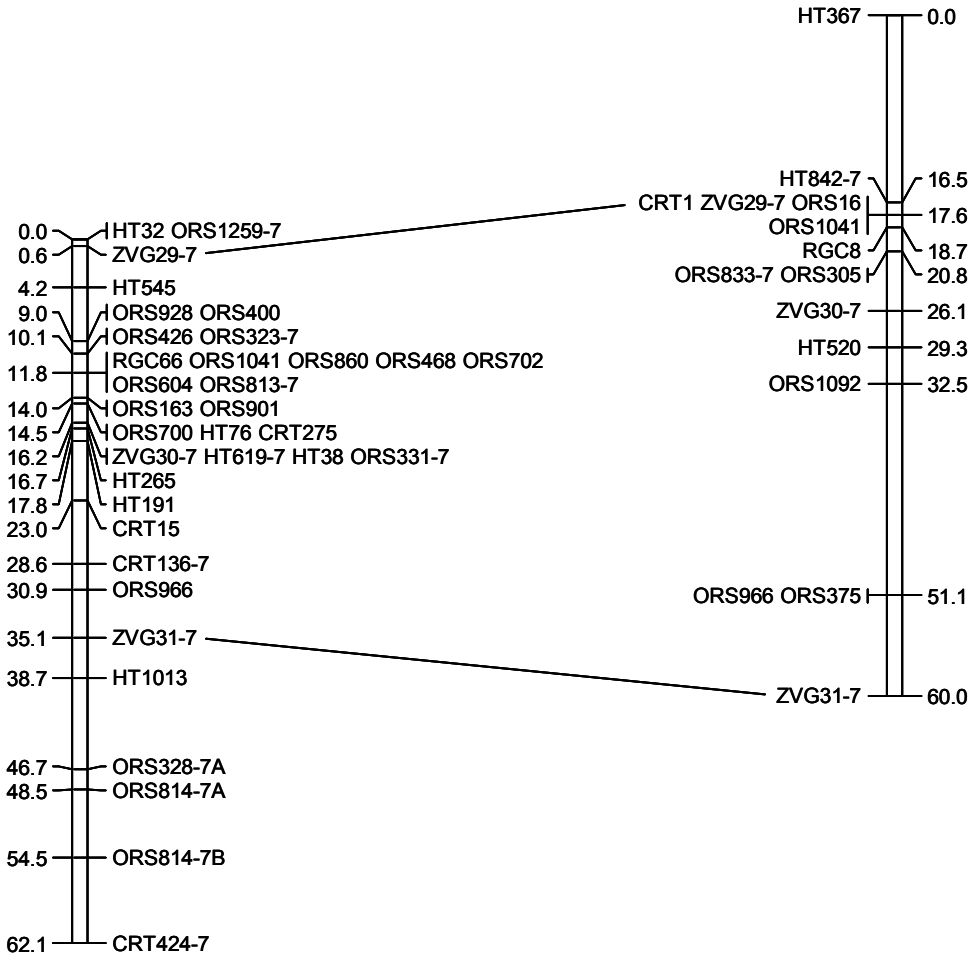
0.6 ORS1193
6.4 HT10
7.5 HT135 HT86
22.0 ORS1229 HT164
22.7 ZVG26-6
25.5 HT18 ORS257-6
26.1 HT534
27.2 HT31
28.3 ORS608 ORS650 ZVG27-6
30.0 HT1
32.3 HT185 ORS516 ORS1219-6
34.0 CRT91 CRT156
36.3 ORS381
36.9 ORS725 ORS1203-6
39.2 HT34 ORS1256 HT92 ORS1233 HT116
CRT373 HT1036 HT1042
ORS230 0.0
ORS1193 *HMGS* ZVG25-6 10.0
HT769 21.9
RGC7 26.9
ORS1229 ZVG26-6 29.4
HT925-6 31.5
ORS1066 34.7
ORS608 36.8
ORS650 45.5
ORS381 50.9
CRT21 59.5
ORS1256 61.6
HT299-6 62.7
ORS483 HT304 ORS339
HT913 *FATB AHAS2* 63.8
ORS934-6
ORS349 65.9

ANN7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4.2 HT545
9.0 ORS928 ORS400
10.1 ORS426 ORS323-7
11.8 RGC66 ORS1041 ORS860 ORS468 ORS702
ORS604 ORS813-7
14.0 ORS163 ORS901
14.5 ORS700 HT76 CRT275
16.2 ZVG30-7 HT619-7 HT38 ORS331-7
16.7 HT265
17.8 HT191
23.0 CRT15
28.6 CRT136-7
30.9 ORS966
35.1 ZVG31-7
38.7 HT1013
46.7 ORS328-7A
48.5 ORS814-7A
54.5 ORS814-7B
62.1 CRT424-7
HT367 0.0
HT842-7 16.5
CRT1 ZVG29-7 ORS16 17.6
ORS1041
RGC8 18.7
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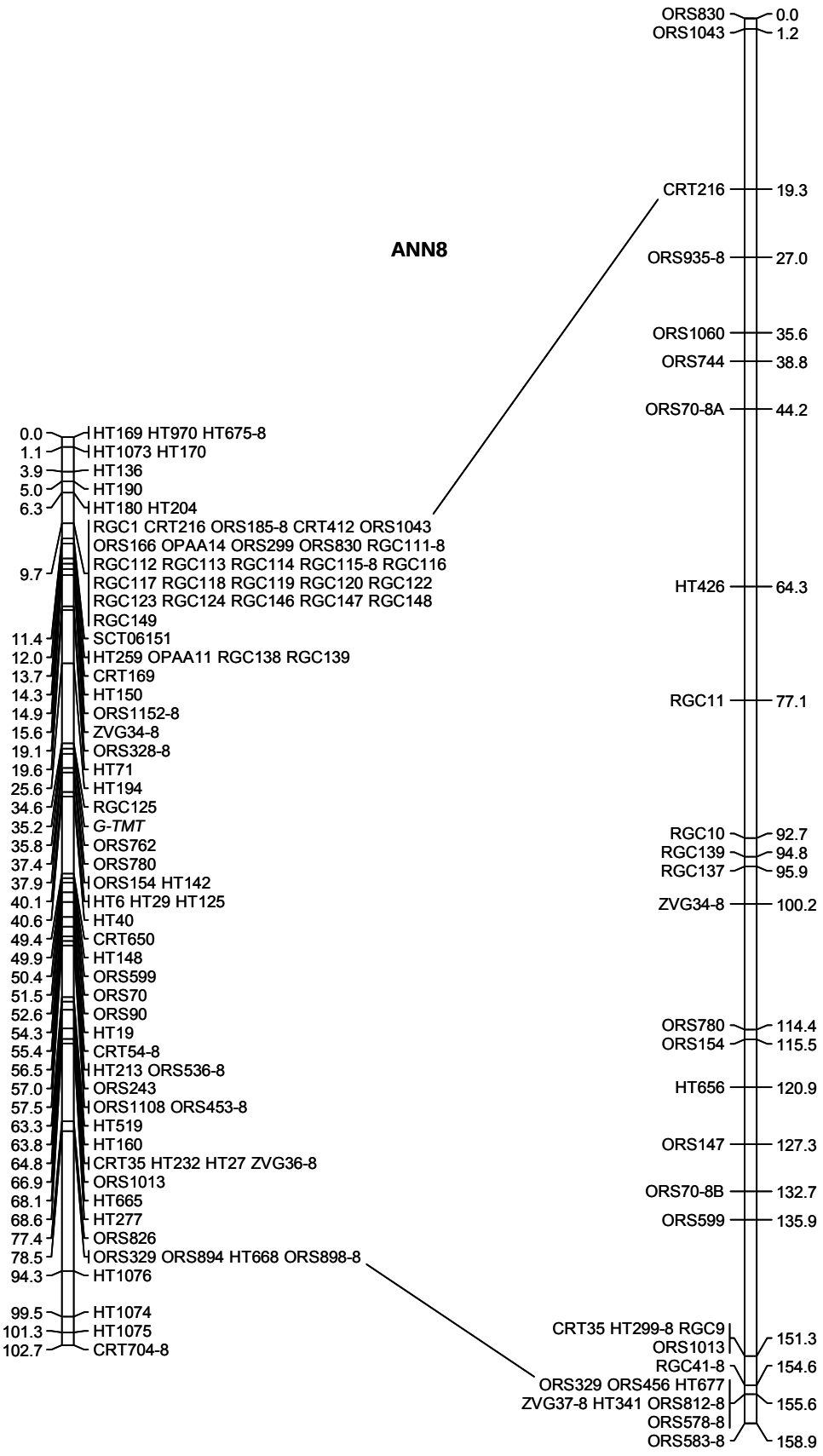
ZVG30-7 26.1
HT520 29.3
ORS1092 32.5
ORS966 ORS375 51.1
ZVG31-7 60.0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1.1 HT1073 HT170
3.9 HT136
5.0 HT190
6.3 HT180 HT204
RGC1 CRT216 ORS185-8 CRT412 ORS1043
ORS166 OPAA14 ORS299 ORS830 RGC111-8
9.7 RGC112 RGC113 RGC114 RGC115-8 RGC116
RGC117 RGC118 RGC119 RGC120 RGC122
RGC123 RGC124 RGC146 RGC147 RGC148
RGC149
11.4 SCT06151
12.0 HT259 OPAA11 RGC138 RGC139
13.7 CRT169
14.3 HT150
14.9 ORS1152-8
15.6 ZVG34-8
19.1 ORS328-8
19.6 HT71
25.6 HT194
34.6 RGC125
35.2 G-TMT
35.8 ORS762
37.4 ORS780
37.9 ORS154 HT142
40.1 HT6 HT29 HT125
40.6 HT40
49.4 CRT650
49.9 HT148
50.4 ORS599
51.5 ORS70
52.6 ORS90
54.3 HT19
55.4 CRT54-8
56.5 HT213 ORS536-8
57.0 ORS243
57.5 ORS1108 ORS453-8
63.3 HT519
63.8 HT160
64.8 CRT35 HT232 HT27 ZVG36-8
66.9 ORS1013
68.1 HT665
68.6 HT277
77.4 ORS826
78.5 ORS329 ORS894 HT668 ORS898-8
94.3 HT1076
99.5 HT1074
101.3 HT1075
102.7 CRT704-8
ORS830 0.0
ORS1043 1.2
CRT216 19.3
ORS935-8 27.0
ORS1060 35.6
ORS744 38.8
ORS70-8A 44.2
HT426 64.3
RGC11 77.1
RGC10 92.7
RGC139 94.8
RGC137 95.9
ZVG34-8 100.2
ORS780 114.4
ORS154 115.5
HT656 120.9
ORS147 127.3
ORS70-8B 132.7
ORS599 135.9
CRT35 HT299-8 RGC9 151.3
ORS1013
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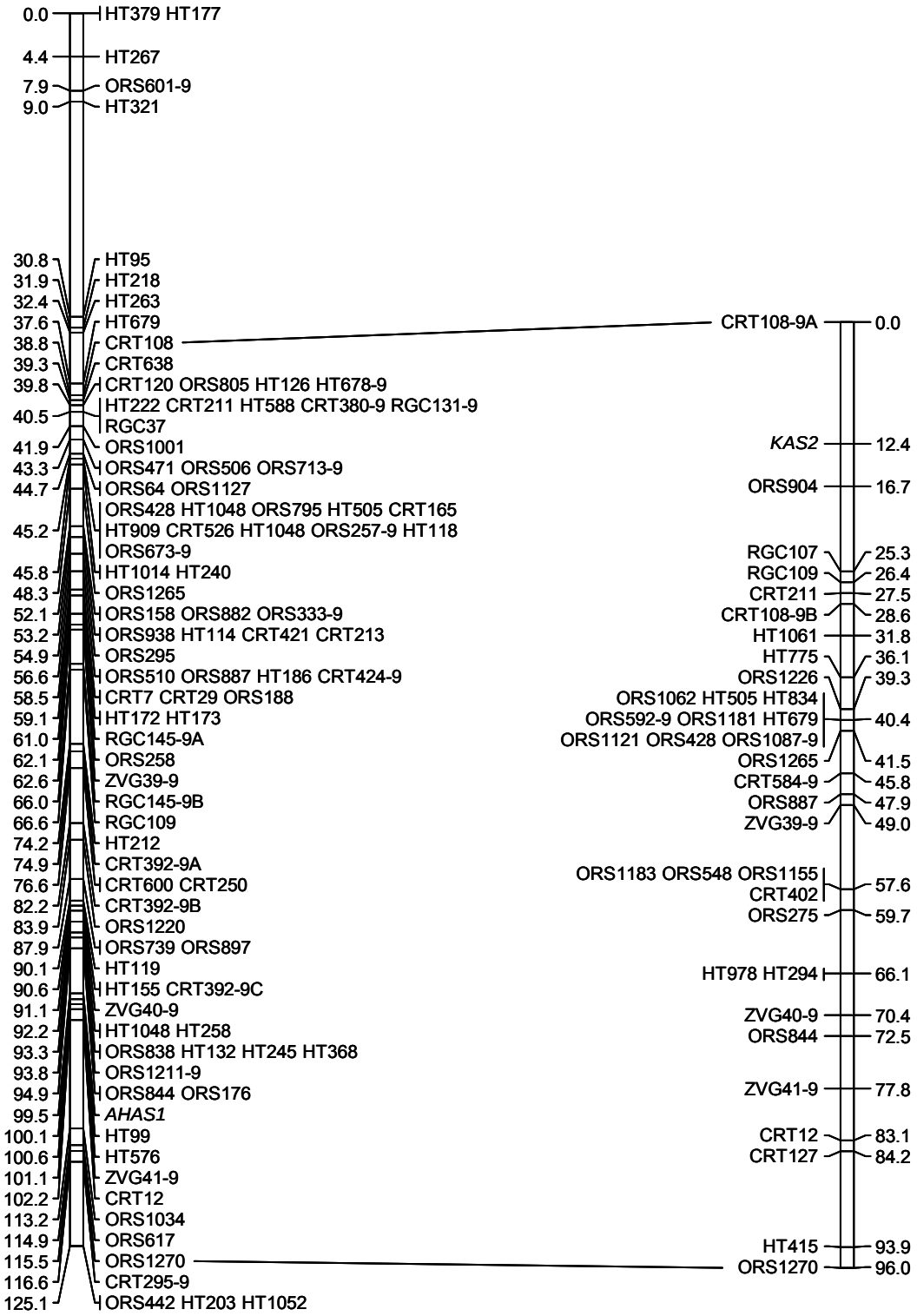
ORS329 ORS456 HT677
ZVG37-8 HT341 ORS812-8 155.6
ORS578-8
ORS583-8 158.9

ANN9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31.9 HT218
32.4 HT263
37.6 HT679
38.8 CRT108
39.3 CRT638
39.8 CRT120 ORS805 HT126 HT678-9
40.5 HT222 CRT211 HT588 CRT380-9 RGC131-9
RGC37
41.9 ORS1001
43.3 ORS471 ORS506 ORS713-9
44.7 ORS64 ORS1127
ORS428 HT1048 ORS795 HT505 CRT165
45.2 HT909 CRT526 HT1048 ORS257-9 HT118
ORS673-9
45.8 HT1014 HT240
48.3 ORS1265
52.1 ORS158 ORS882 ORS333-9
53.2 ORS938 HT114 CRT421 CRT213
54.9 ORS295
56.6 ORS510 ORS887 HT186 CRT424-9
58.5 CRT7 CRT29 ORS188
59.1 HT172 HT173
61.0 RGC145-9A
62.1 ORS258
62.6 ZVG39-9
66.0 RGC145-9B
66.6 RGC109
74.2 HT212
74.9 CRT392-9A
76.6 CRT600 CRT250
82.2 CRT392-9B
83.9 ORS1220
87.9 ORS739 ORS897
90.1 HT119
90.6 HT155 CRT392-9C
91.1 ZVG40-9
92.2 HT1048 HT258
93.3 ORS838 HT132 HT245 HT368
93.8 ORS1211-9
94.9 ORS844 ORS176
99.5 AHAS1
100.1 HT99
100.6 HT576
101.1 ZVG41-9
102.2 CRT12
113.2 ORS1034
114.9 ORS617
115.5 ORS1270
116.6 CRT295-9
125.1 ORS442 HT203 HT1052
CRT108-9A 0.0
KAS2 12.4
ORS904 16.7
RGC107 25.3
RGC109 26.4
CRT211 27.5
CRT108-9B 28.6
HT1061 31.8
HT775 36.1
ORS1226 39.3
ORS1062 HT505 HT834
ORS592-9 ORS1181 HT679 40.4
ORS1121 ORS428 ORS1087-9
ORS1265 41.5
CRT584-9 45.8
ORS887 47.9
ZVG39-9 49.0
ORS1183 ORS548 ORS1155
CRT402 57.6
ORS275 59.7
HT978 HT294 66.1
ZVG40-9 70.4
ORS844 72.5
ZVG41-9 77.8
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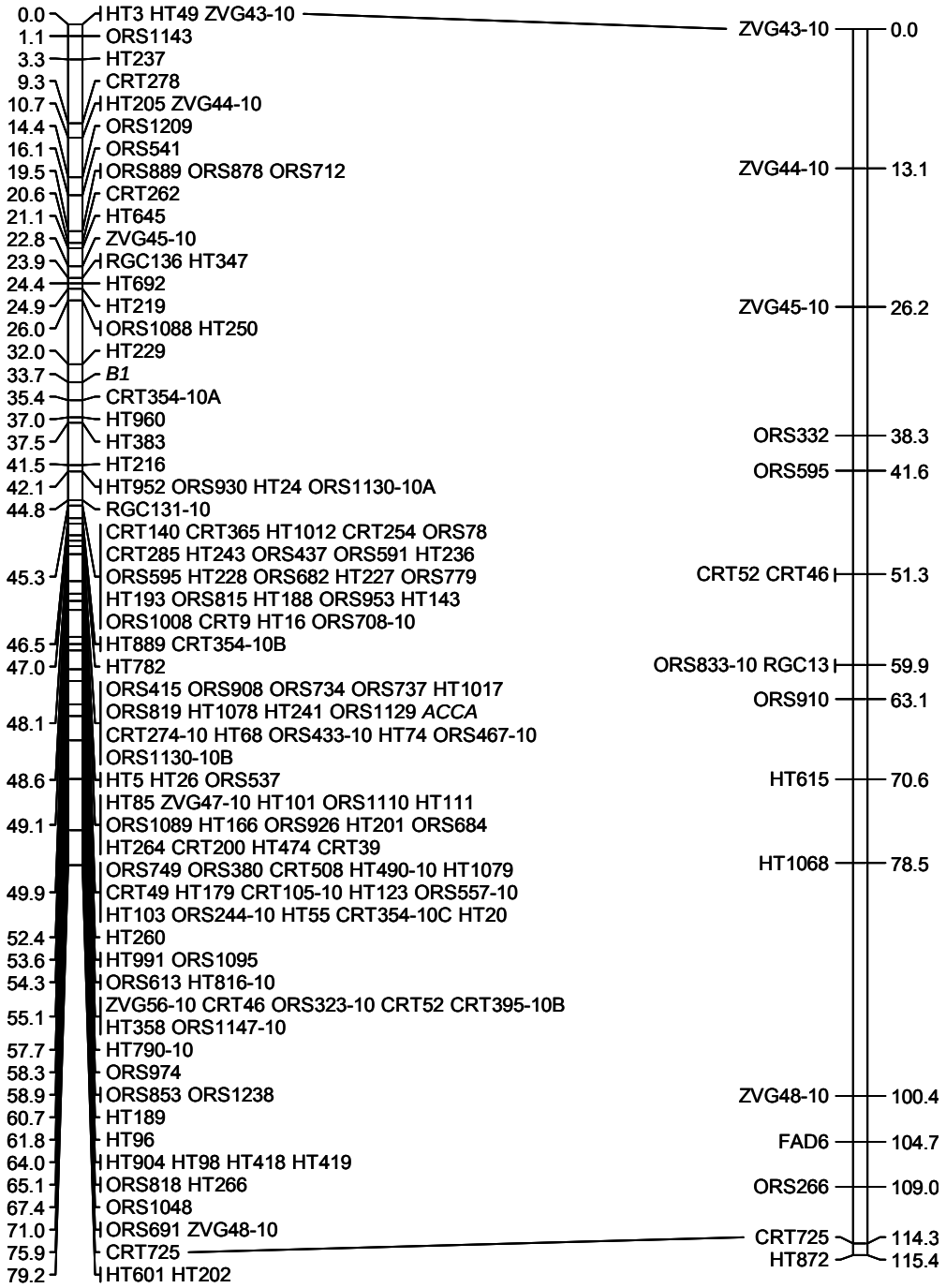
CRT127 84.2
HT415 93.9
ORS1270 96.0

ANN10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3.3 HT237
9.3 CRT278
10.7 HT205 ZVG44-10
14.4 ORS1209
16.1 ORS541
19.5 ORS889 ORS878 ORS712
20.6 CRT262
21.1 HT645
22.8 ZVG45-10
23.9 RGC136 HT347
24.4 HT692
24.9 HT219
26.0 ORS1088 HT250
32.0 HT229
33.7 B1
35.4 CRT354-10A
37.0 HT960
37.5 HT383
41.5 HT216
42.1 HT952 ORS930 HT24 ORS1130-10A
44.8 RGC131-10
45.3 CRT140 CRT365 HT1012 CRT254 ORS78
CRT285 HT243 ORS437 ORS591 HT236
ORS595 HT228 ORS682 HT227 ORS779
HT193 ORS815 HT188 ORS953 HT143
ORS1008 CRT9 HT16 ORS708-10
46.5 HT889 CRT354-10B
47.0 HT782
48.1 ORS415 ORS908 ORS734 ORS737 HT1017
ORS819 HT1078 HT241 ORS1129 ACCA
CRT274-10 HT68 ORS433-10 HT74 ORS467-10
ORS1130-10B
48.6 HT5 HT26 ORS537
49.1 HT85 ZVG47-10 HT101 ORS1110 HT111
ORS1089 HT166 ORS926 HT201 ORS684
HT264 CRT200 HT474 CRT39
49.9 ORS749 ORS380 CRT508 HT490-10 HT1079
CRT49 HT179 CRT105-10 HT123 ORS557-10
HT103 ORS244-10 HT55 CRT354-10C HT20
52.4 HT260
53.6 HT991 ORS1095
54.3 ORS613 HT816-10
55.1 ZVG56-10 CRT46 ORS323-10 CRT52 CRT395-10B
HT358 ORS1147-10
57.7 HT790-10
58.3 ORS974
58.9 ORS853 ORS1238
60.7 HT189
61.8 HT96
64.0 HT904 HT98 HT418 HT419
65.1 ORS818 HT266
67.4 ORS1048
71.0 ORS691 ZVG48-10
75.9 CRT725
79.2 HT601 HT202
ZVG43-10 0.0
ZVG44-10 13.1
ZVG45-10 26.2
ORS332 38.3
ORS595 41.6
CRT52 CRT46 51.3
ORS833-10 RGC13 59.9
ORS910 63.1
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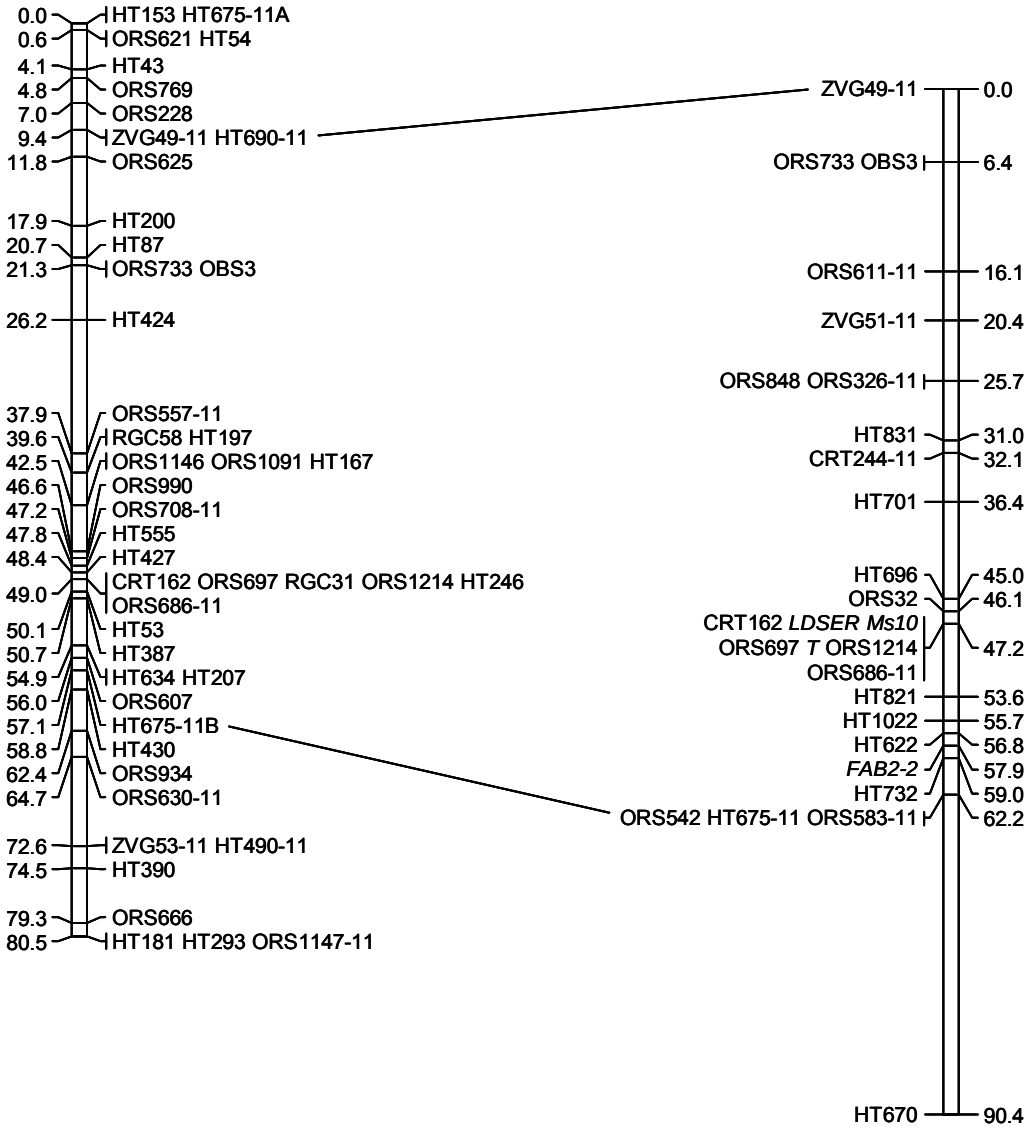
ORS266 109.0
CRT725 114.3
HT872 115.4

ANN11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4.8 ORS769
7.0 ORS228
9.4 ZVG49-11 HT690-11
11.8 ORS625
17.9 HT200
20.7 HT87
21.3 ORS733 OBS3
26.2 HT424
37.9 ORS557-11
39.6 RGC58 HT197
42.5 ORS1146 ORS1091 HT167
46.6 ORS990
47.2 ORS708-11
47.8 HT555
48.4 HT427
49.0 CRT162 ORS697 RGC31 ORS1214 HT246
ORS686-11
50.1 HT53
50.7 HT387
54.9 HT634 HT207
56.0 ORS607
57.1 HT675-11B
58.8 HT430
62.4 ORS934
64.7 ORS630-11
72.6 ZVG53-11 HT490-11
74.5 HT390
79.3 ORS666
80.5 HT181 HT293 ORS1147-11
ZVG49-11 0.0
ORS733 OBS3 6.4
ORS611-11 16.1
ZVG51-11 20.4
ORS848 ORS326-11 25.7
HT831 31.0
CRT244-11 32.1
HT701 36.4
HT696 45.0
ORS32 46.1
CRT162 LDSER Ms10
ORS697 T ORS1214 47.2
ORS686-11
HT821 53.6
HT1022 55.7
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FAB2-2 57.9
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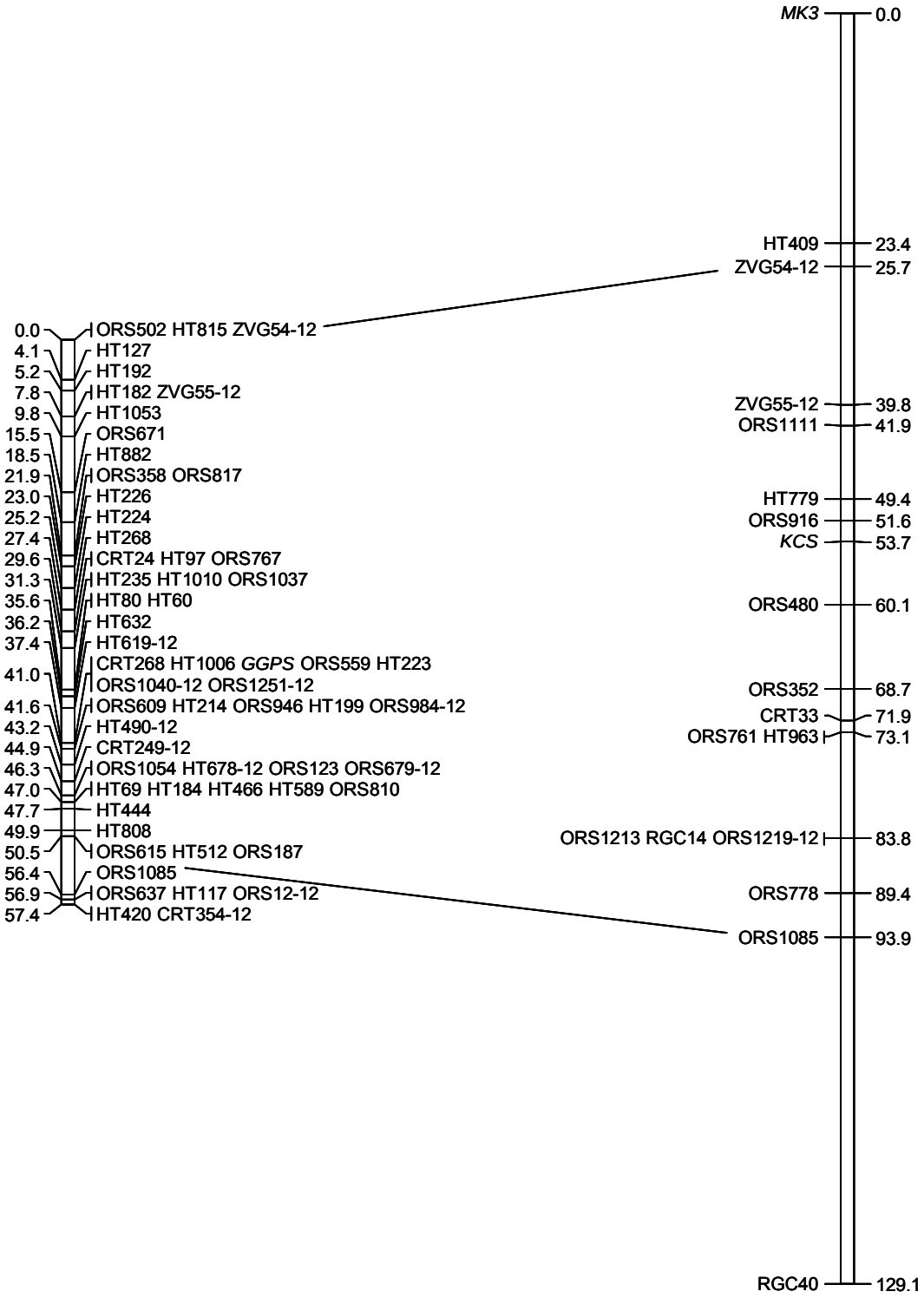
ORS542 HT675-11 ORS583-11 62.2
HT670 90.4

ANN12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5.2 HT192
7.8 HT182 ZVG55-12
9.8 HT1053
15.5 ORS671
18.5 HT882
21.9 ORS358 ORS817
23.0 HT226
25.2 HT224
27.4 HT268
29.6 CRT24 HT97 ORS767
31.3 HT235 HT1010 ORS1037
35.6 HT80 HT60
36.2 HT632
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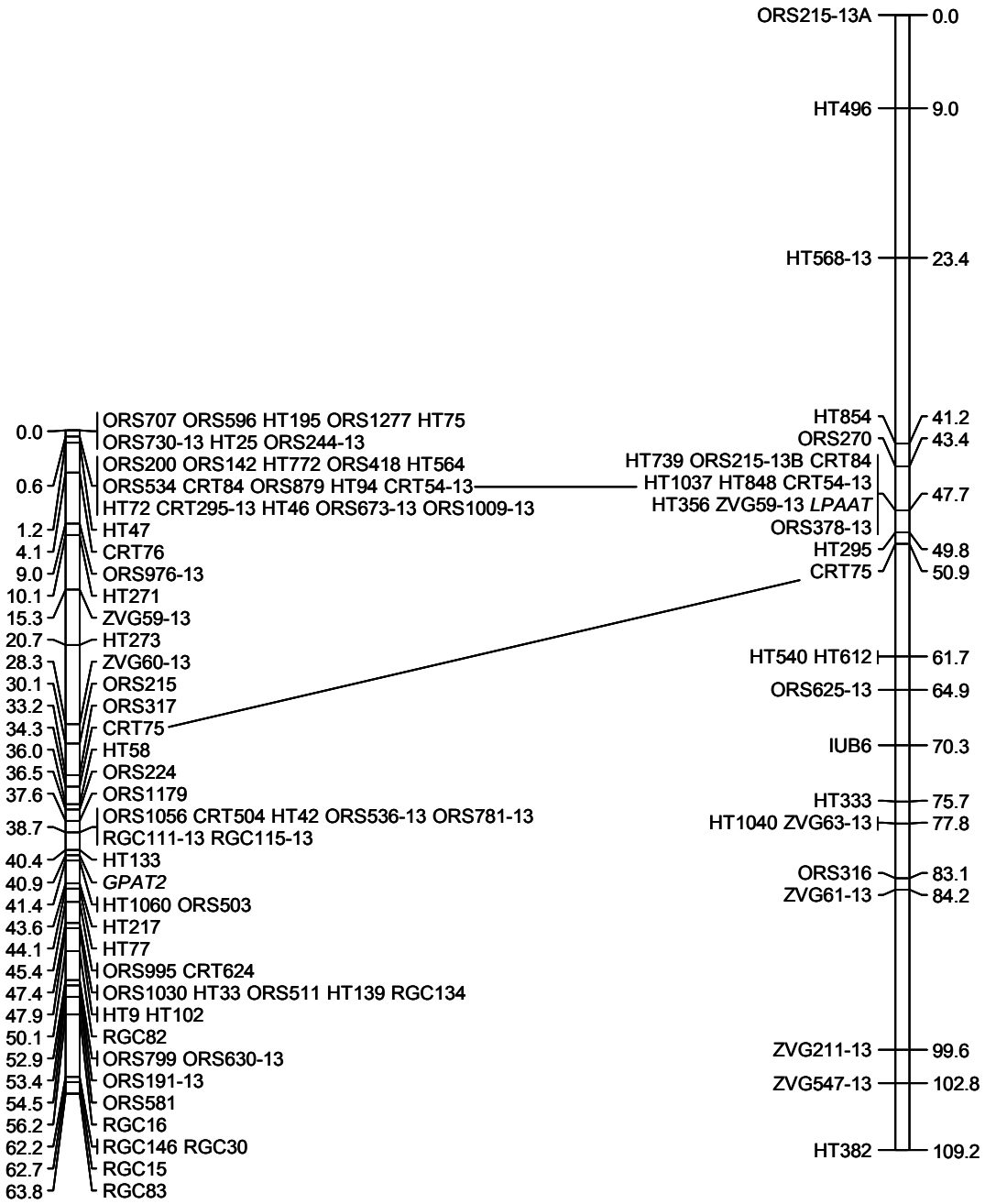
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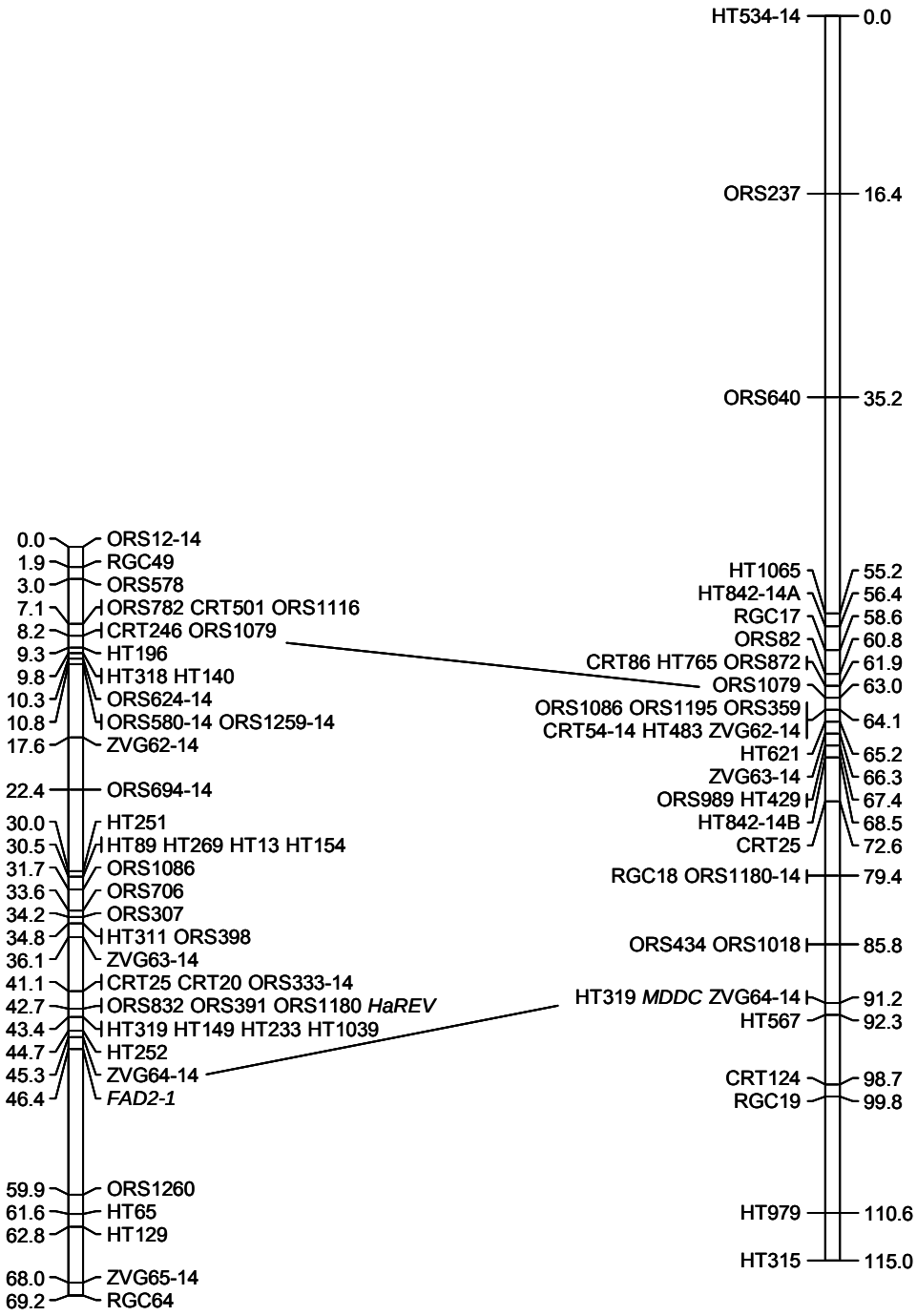
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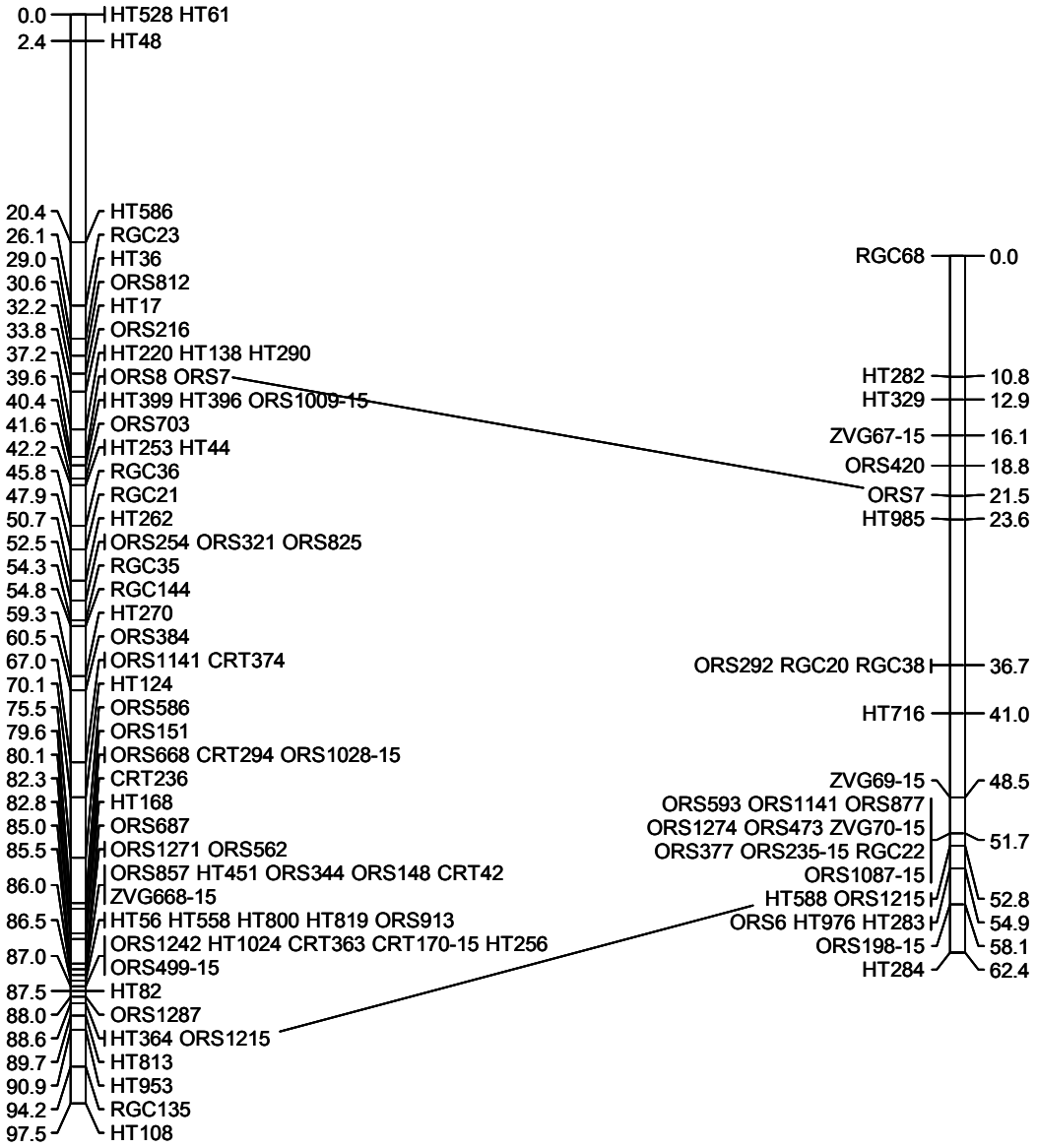
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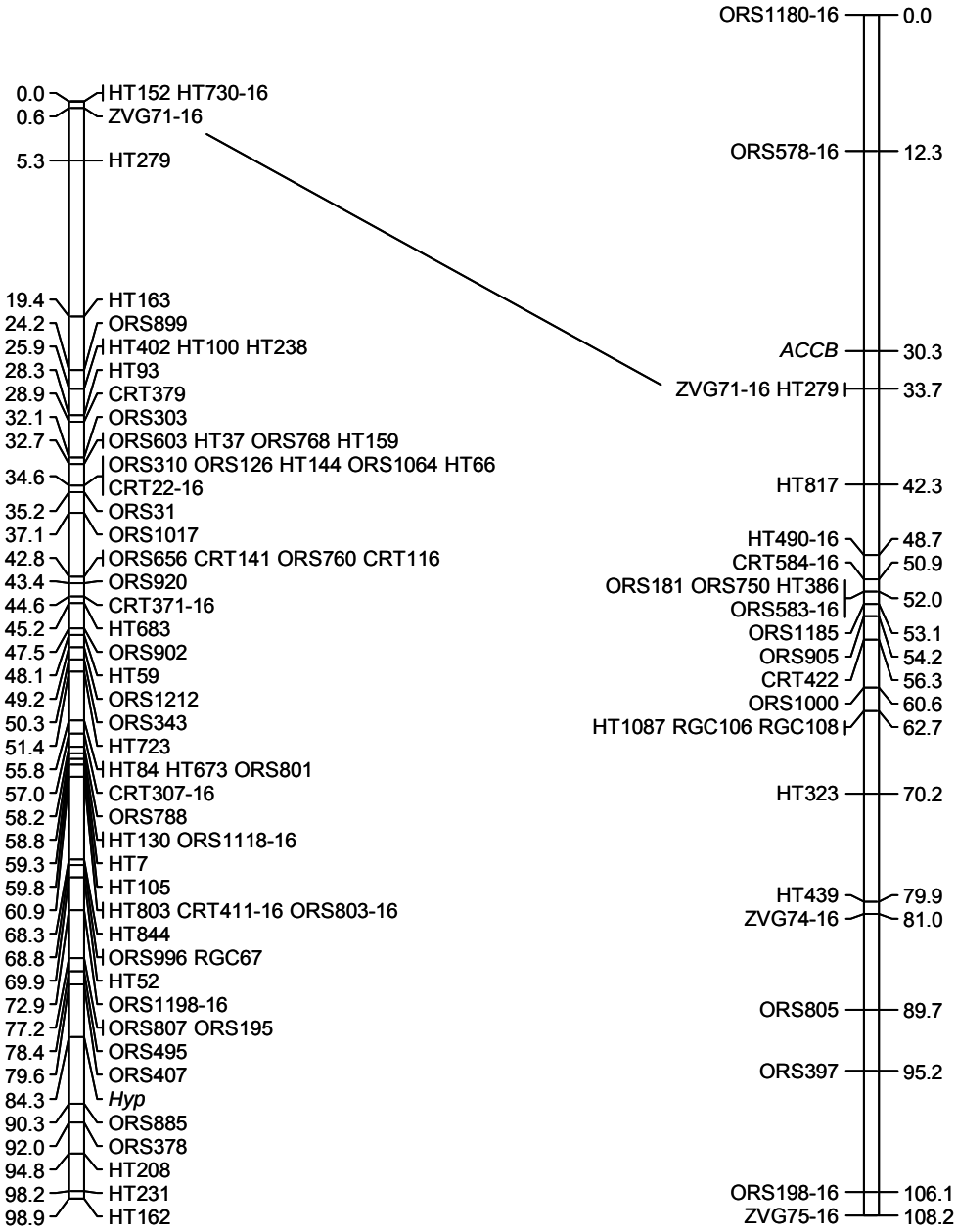
ANN16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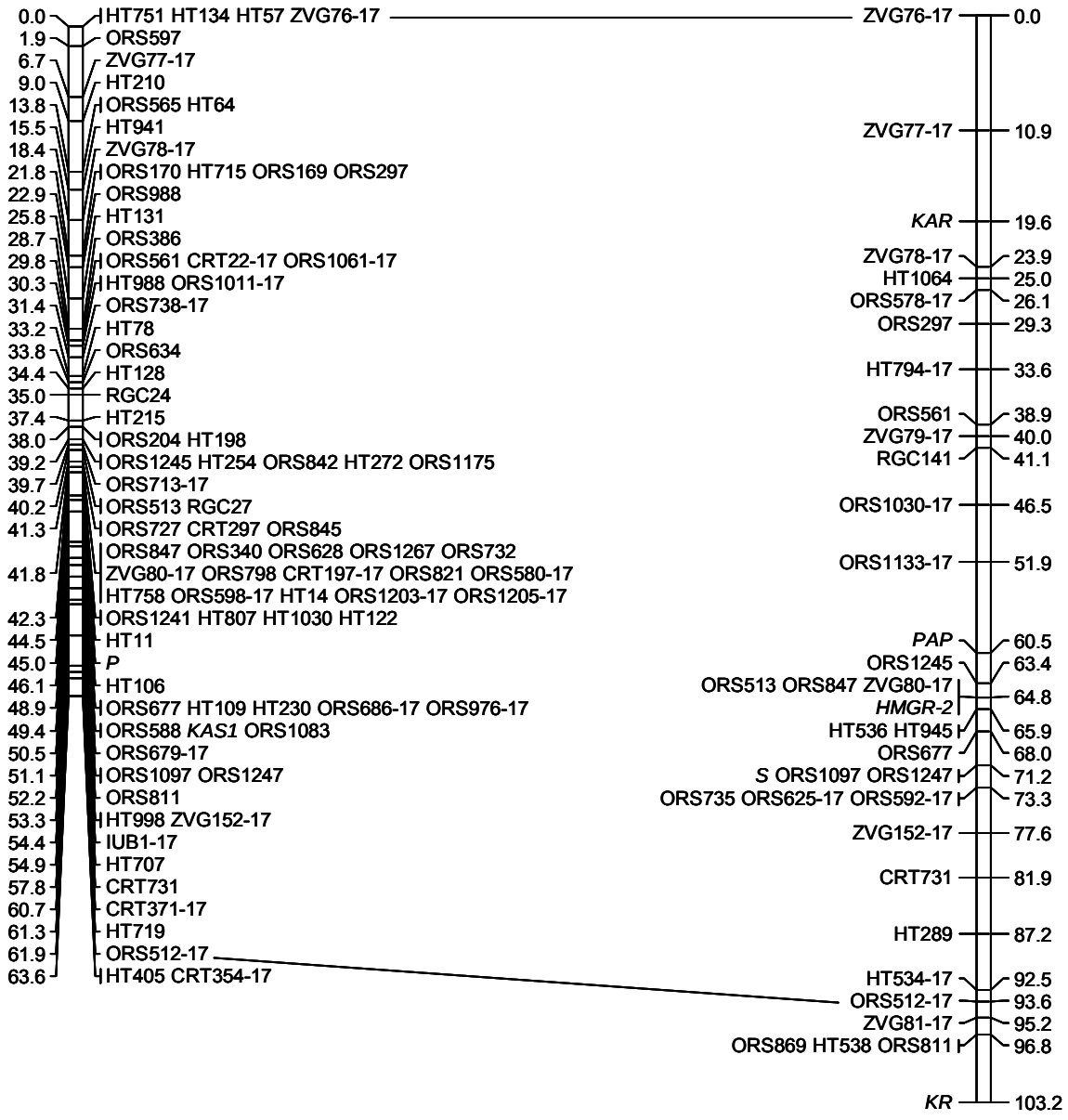
ANN17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