CONSTRUCTION OF A HIGH DENSITY, COMPOSITE RFLP LINKAGE MAP FOR CULTIVATED SUNFLOWER (*Helianthus annuus* L.).

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Abstract
A high density, composite RFLP linkage map for the sunflower genome was constructed based on the segregation of 635 marker loci, detected by 500 probes, in 9 different F_2 populations. The genetic markers covered 1472 centiMorgans (cM) of the sunflower genome and were arranged in 17 linkage groups (A to Q), corresponding to the haploid number of chromosomes in this species. Three loci remained unlinked, but two of these markers were tightly linked to each other. Although the average interval size was 2.3 cM, there were still a number of persistent gaps (i.e. > 20 cM) as well as areas of acute marker clustering, some of which probably correspond to the location of centromeres. The high level of gene duplication within the sunflower genome is consistent with the hypothesised evolution of *H. annuus* through allopolyploidy; however homoeologous linkage groups could not be identified.

Key words
Restriction fragment length polymorphisms (RFLPs) - Linkage map - Composite RFLP map - *Helianthus annuus* - Sunflower

Introduction
Cultivated sunflower (*Helianthus annuus* L.) is a diploid, allogamous annual (2n=2x=34) which is grown primarily for its seed oil throughout the warm temperate regions of the five continents. The annual world production of sunflower oil is over 200 million tonnes per annum, which is currently third behind soybean and cottonseed oils. Despite the economic importance of sunflower, the application of biotechnology to this crop has been extremely limited. Recently, however, the use of restriction fragment length polymorphisms (RFLPs) as genetic markers has been reported in sunflower (BERRY *et al.* 1994; GENTZBITTEL *et al.* 1994) and these studies revealed that there is a high level of DNA polymorphism in the cultivated germplasm pool. This finding has allowed the rapid construction of the first RFLP linkage maps for sunflower (BERRY *et al.* 1995; GENTZBITTEL *et al.* 1995) which have already been used to identify both qualitative (MOUZEYAR *et al.* 1995, LEON *et al.* in press) and quantitative trait loci (LEON *et al.* 1995). The two published sunflower maps differ in that the one described by BERRY *et al.* (1995) is based on a single F_2 mapping population, whereas the one reported by GENTZBITTEL *et al* (1995) is a composite map produced from three F_2 and two BC_1 populations. The use of a single intraspecific population (ZENB8xHA89) only allowed BERRY *et al.* (1995) to map 55% of the available sunflower RFLP probes. In this paper we describe the addition of 400 new loci to the ZENB8xHA89 map using the segregation data from another 8 F_2 populations and the computer program JoinMap (STAM 1993) in order to produce a single composite map.

Materials and methods
The methods for RFLP analysis and linkage map construction are described by BERRY *et al.* (1994 and 1995). Six hundred and twelve low-copy probes selected from the cDNA (582) and *PstI* (30) genomic libraries described by BERRY *et al.* (1994) were radioactively-labelled and hybridised to Southern blots of the 13 inbred lines that were used to make the 9 F_2 mapping populations (Table 1). Probes that were identified as being polymorphic in a given population were then hybridised to Southern blots of F_2 DNA and the data scored and analysed according to BERRY *et al.* (1995). In addition to the RFLP loci, one isozyme marker (*Pgd-3*) was mapped on the ZENB8xHA89 F_2 according to CARRERA *et al.* (1995), as well as the dominant gene (*Hyp*) controlling white pigmentation of the hypodermis seed layer (LEON *et al.* in press).

Table 1. The number of individuals and RFLP loci mapped in each of the nine F_2 populations used to construct the sunflower composite map.

Population	No. of plants	No. of loci mapped
ZENB8xHA89	289	250
ZENB8xPAC2	70	334
ZENB8xZENR7	142	91
BSA52xRHA297	58	114
HA89xRHA271	94	118
ZENR7xRHA801	94	93
HA89xZENR9	115	38
ZENR1xZENR8	207	103
ZENB4xHA300	218	152

Linkage maps for each of the nine individual F_2 populations were constructed using MAPMAKER version 3.0 (LANDER *et al.* 1987). The composite map was produced using JoinMap (STAM 1993) by analysing the RFLP data on a group by group basis, using the default LOD score of 0.05 and the Kosambi mapping function. The integration of distinct genetic linkage maps requires the use of at least two common markers per linkage group between any two populations. These markers then act as anchor points allowing the merging of linkage groups in the correct orientation from the different crosses.

Results and discussion

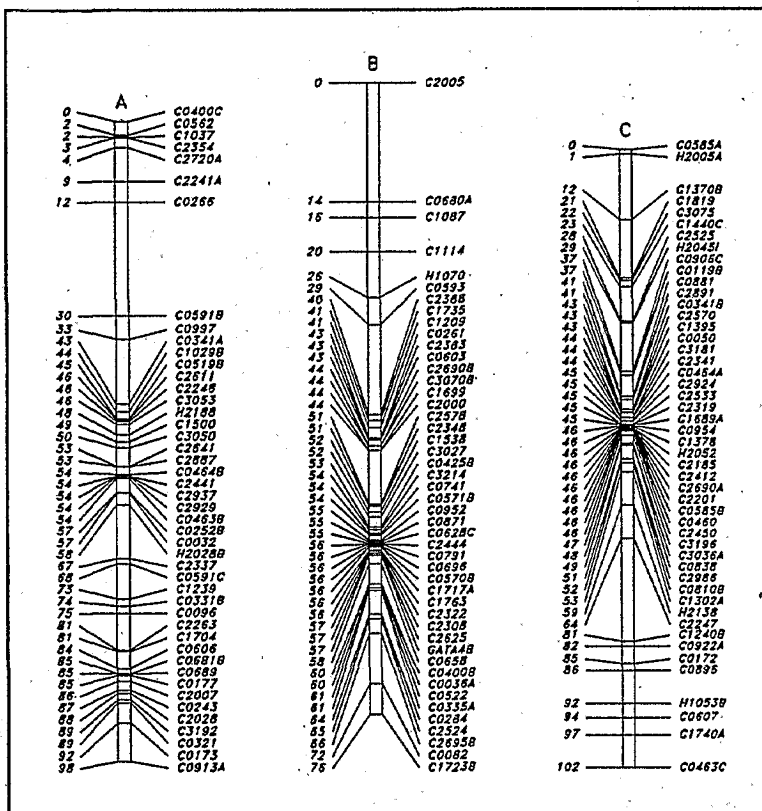
To-date 500 of the available 612 low-copy RFLP probes have been mapped on at least one of the nine F_2 populations listed in Table 1. These 500 probes detected 633 different loci within the sunflower genome, the majority of which were scored as codominant markers and followed the expected 1:2:1 segregation ratio. However, in each F_2 population there were regions of distorted segregation which were caused by a reduction in one or other of the parental homozygous classes (data not shown). Distorted segregation is a common phenomenon in the published plant RFLP maps and it is thought to be caused by selection against individual genotypes during either sporogenesis, gametogenesis, seed development, germination or plant growth. The 633 RFLP loci, plus the *Pgd-3* and *Hyp* loci, were arranged into 17 linkage groups (Figure 1), which probably correspond to the 17 haploid chromosomes of *H. annuus*. The letter used here to identify each group (A to Q) is completely arbitrary as no classical genetic map exists for sunflower. The composite map shown in Figure 1 covers 1472 cM of the sunflower genome and represents the most complete and saturated genetic map available for *H. annuus*. The process of consensus mapping has effectively added a further 400 loci to the ZENB8xHA89 map, which covered 1380 cM and was thought to represent 60-80% of the sunflower genome (BERRY *et al.* 1995). It is now clear that this earlier estimate of genome coverage was too conservative and, in fact, the composite map covers approximately 90% of the genome based on an estimated total genome length of 1650 cM (GENTZBITTEL *et al.* 1995). The independent segregation of 3 loci (C0592A and C1370A which are 2 cM apart and H2028A) also confirms that at least 100 cM of the sunflower genome are still missing.

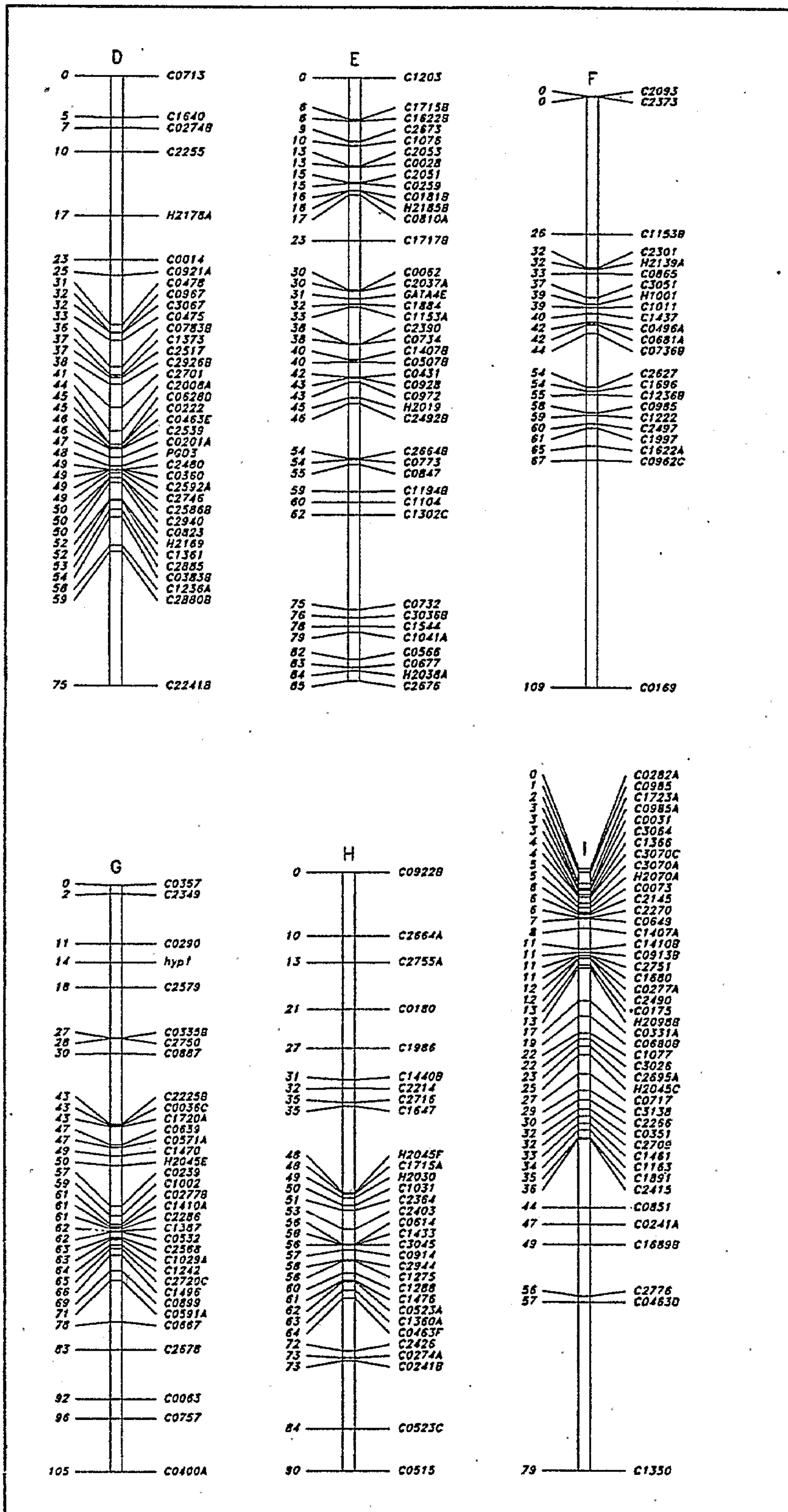
Due to the variation in recombination frequency across the genome, the distribution of markers within linkage groups is far from uniform. For example, large gaps of greater than 20 cM are present on linkage groups D, F, I, M and O (Figure 1). Gaps such as these are common in the published plant RFLP linkage maps and either represent regions of high recombination or reflect an under-representation of clones from these areas in the libraries used as probe sources. As well as regions devoid of marker loci, there are also areas of acute marker clustering such as those seen on linkage groups B, C and J (Figure 1). In some plant species these areas of reduced recombination have been found to correspond to the position of the centromere. In general, the ordering of marker loci in the composite map is fairly consistent with the individual F_2 maps (data not shown); however the exact order within these tight clusters of marker loci is uncertain.

The presence of 133 duplicated loci on the linkage map supports the hypothesis that *H. annuus* has evolved through allopolypoidy via the hybridisation of two *Viguiera* progenitor species of n=8 and n=9 (HEISER and

SMITH 1955); however there is no apparent conservation of linkage blocks between homoeologous chromosomes as seen in the RFLP linkage maps of other allopolyploids, such as maize (HELENTJARIS *et al.* 1988). The sunflower genome may be similar to that of soybean where the collinearity between the progenitor genomes is being lost through the process of diploidisation (ZHU et al. 1994). An alternative hypothesis is that a large number of the independent duplications within the sunflower genome are due to pseudogene families (GENTZBITTEL *et al.* 1995). In fact, the *Pst*I clone H2045, which mapped to 9 unlinked loci, is part of the reverse transcriptase gene from a *copia*-like retrotransposon (unpublished data). The presence of such elements in the sunflower genome would allow the reverse transcription of endogenous mRNA to take place and hence the formation of pseudogenes.

Figure 1. A composite sunflower linkage map of 633 RFLP loci identified by 500 low-copy DNA probes. The 17 linkage groups are listed at the *top*, the loci are listed on the *right* and the cumulative map distances in centiMorgans (cM) are shown on the *left*. Loci nomenclature is described by BERRY *et al.* (1995). The *Pgd-3* and *Hyp* loci map to linkage groups D and G respectively.

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33 C0475
36 C0783B
37 C1373
37 C2517
38 C2926B
41 C2701
44 C2008A
45 C0628D
45 C0222
46 C0463E
46 C2539
47 C0201A
48 PG03
49 C2480
49 C0360
49 C2592A
49 C2746
50 C2586B
50 C2940
50 C0823
52 H2169
52 C1361
53 C2885
54 C0383B
58 C1236A
59 C2880B
75 C2241B
E
0 C1203
6 C1715B
6 C1622B
9 C2673
10 C1076
13 C2053
13 C0028
15 C2051
15 C0259
16 C0181B
16 H2185B
17 C0810A
23 C1717B
30 C0062
30 C2037A
31 GATA4E
32 C1884
33 C1153A
38 C2390
38 C0734
40 C1407B
40 C0507B
42 C0431
43 C0928
43 C0972
45 H2019
46 C2492B
54 C2664B
54 C0773
55 C0847
59 C1194B
60 C1104
62 C1302C
75 C0732
76 C3036B
78 C1544
79 C1041A
82 C0566
83 C0677
84 H2038A
85 C2676
F
0 C2093
0 C2373
26 C1153B
32 C2301
32 H2139A
33 C0865
37 C3051
39 H1001
39 C1011
40 C1437
42 C0496A
42 C0681A
44 C0736B
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67 C0962C
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27 C0335B
28 C2750
30 C0887
43 C2225B
43 C0036C
43 C1720A
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62 C0532
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64 C1242
65 C2720C
66 C1496
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83 C2678
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27 C1986
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35 C1647
48 H2045F
48 C1715A
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56 C3045
57 C0914
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61 C1476
62 C0523A
63 C1360A
64 C0463F
72 C2426
73 C0274A
73 C0241B
84 C0523C
90 C0515
I
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2 C1723A
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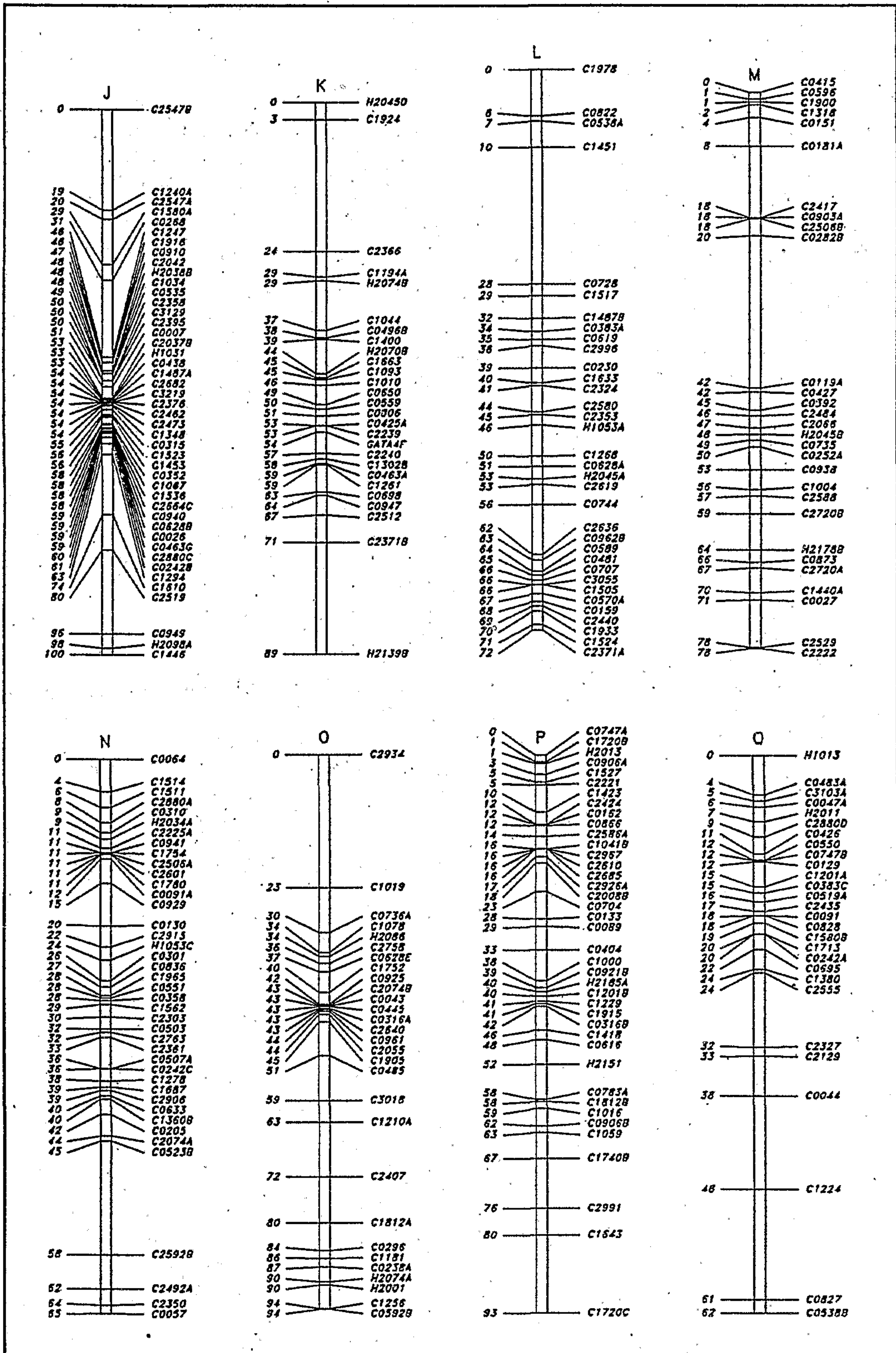
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49 C0535
50 C2358
50 C3125
50 C2395
51 C0007
53 C2037B
53 H1031
53 C0438
54 C1487A
54 C2682
54 C3219
54 C2376
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54 C2473
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55 C0315
56 C1523
56 C1453
58 C0352
58 C1087
58 C1336
58 C2564C
59 C0940
59 C0628B
59 C0026
59 C0463C
60 C2880C
61 C0242B
63 C1294
74 C1610
80 C2519
96 C0949
98 H2098A
100 C1446
K
0 H2045D
3 C1924
24 C2366
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29 H2074B
37 C1044
38 C0496B
39 C1400
44 H2070B
45 C1663
45 C1093
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