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Mapping genetic factors controlling pollen viability in an interspecific cross in *Helianthus* sect. *Helianthus*

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Abstract Segregation of 48 genetic markers, including one CMS restorer gene, one morphological character gene, six isozymes and 40 RAPD loci, was scored in a backcross progeny of an interspecific hybrid *H. argophyllus*×*H. annuus* cv RHA274. A linkage map was generated taking into account segregation distortions for 11 of the 48 loci in the frame of two different models considering locus-pair segregation in the context of either independent selection pressures or non-equilibrated parental classes. The map consists of nine linkage groups and nine isolated markers covering 390 cM. Approximately half of the plants of the BC1 were male fertile as expected for the segregation of one dominant male-fertility restorer gene; however, these displayed a large range of variation for pollen viability. About 80% of this variation was explained by three genomic regions located on linkage groups 1, 2 and 3. The observation of meiotic chromosomes revealed a significant rate of mispairing (rod bivalents and tetravalents) in tight correlation with pollen viability, indicating that chromosome rearrangements (translocations) are the preponderant factors reducing pollen viability in this progeny. Cytogenetic and mapping data suggest that the three genomic regions involved in pollen-viability variation are located close to translocation points which differentiate the parental-species karyotypes. Segregation distortion was observed for loci correlated with pollen-viability variation. These were most likely the result of two possible suggested mechanisms.

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Introduction

To utilise genetic resources in breeding programs, it is important to better understand the reproductive barriers between wild and cultivated species. Several *Helianthus* species are of great interest for the genetic improvement of the cultivated sunflower, *Helianthus annuus* var. *macrocarpus* L. (Seiler 1988). Interspecific progenies could serve as an important variability resource. Unfortunately little is known about the fertility and recombination efficiency with cultivated sunflower, or of their consequences for the maintenance of variability.

The cultivated sunflower and 11 annual species belong to the section *Helianthus* of the genus *Helianthus*. These species and *H. annuus* L. are diploid (2n=34), obligatory out-crossing, and self-incompatible. The karyotypes of species belonging to sect. *Helianthus* differ by a variable number of reciprocal translocations and paracentric inversions (Whelan 1978; Chandler et al. 1986). These chromosomal rearrangements are generally well correlated with the pollen fertility of first-generation interspecific hybrids (Chandler et al. 1986) and have been reported to be the preponderant causal factor of reproductive barriers between the species of sect. *Helianthus*.

Helianthus argophyllus T. & G. is closely related to wild *H. annuus* L. (Heiser et al. 1969). The two species display common morphological characteristics such as general plant architecture and large leaves, in contrast to other annual species with small leaves. According to Chandler et al. (1986), *H. argophyllus* and *H. annuus* karyotypes differ only by two reciprocal translocations. Interspecific hybrids are highly vigorous, but female and male fertilities are reduced. From a breeding point of view, *H. argophyllus* contains several disease-resistance factors (Rogers et

al. 1982; Skoric 1985; Griveau et al. 1992, Quresh et al. 1993), and is a source for cytoplasmic male-sterility and male-fertility restoration genes (Christov 1990; Serieys 1994). This species also displays xerophytic traits which should confer drought tolerance to the sunflower (Gelfi and Blanchet 1980; Serieys 1991). Several genetic pools have been derived from interspecific hybrids between *H. annuus* and *H. argophyllus* in order to introgress wild traits into the cultivated background (Morizet et al. 1984; Miller et al. 1992; Seiler 1992).

However, the role of reproductive barriers has not been studied and, furthermore, correlations between the quantitative contribution of the *H. argophyllus* genome to these pools and to the recombination frequency have not been examined. With molecular markers, QTLs controlling wild traits of agronomic interest can be determined. In addition, loci which correlate with such traits as sterility can be identified provided that a map is available for the appropriate crosses.

To address these problems, we have constructed a genetic linkage map in a backcross population derived from *H. annuus* and *H. argophyllus*, and identified genetic factors limiting the pollen viability of interspecific BC1 plants. Pollen viability has been analysed in the light of cytogenetic data from this BC1 progeny.

Material and methods

Plant material

The genetic map was constructed on the basis of 133 backcross (BC1) progeny obtained from the cross [*H. annuus* cv PEF1-RHA274× (*H. argophyllus*×*H. annuus* cv RHA274)]. The *H. argophyllus* parent was a unique plant from the accession FRAINRAMPG no.92 (IBPGR code). This accession is a late-flowering type from Texas. The USDA inbred line RHA274 was used as the male parent to produce F1 interspecific plants. The BC1 population was obtained by crossing a unique F1 interspecific hybrid plant onto the cytoplasmic male-sterile (CMS) line PEF1-RHA274. This line is an alloplasmic version of RHA274 obtained from *H. petiolaris fallax* (Serieys and Vincourt 1987). RHA274 maintains the PEF1-CMS whereas *H. argophyllus* accession no. 92 restores it. BC1 progeny and ten plants of each parental genotype (RHA274 and *H. argophyllus*×RHA274) were sown in a greenhouse. They were transplanted in the field at the first-leaf stage.

The relation between pollen viability and chromosome-end arrangements at meiosis was evaluated on 15 BC1 male-fertile plants which were full sibs of the ones used in the linkage map. These BC1 plants were sampled to represent variability for pollen viability in the mapping population. Cytogenetic analyses were performed on parental genotypes RHA274, *H. argophyllus*, and *H. argophyllus*×RHA274.

Pollen viability determination

Pollen viability (i.e. non-aborted pollen) was estimated according to the staining procedure of Alexander (1969) on the parental genotypes, the 74 restored male-fertile BC1 plants (mapping population), and an additional 15 BC1 plants (cytogenetic analysis). For each plant, three samples of fresh pollen were harvested on different heads the day before anthesis. Pollen viability was noted as the percentage of stained nuclei, on a minimum of 300 pollen grains per sample.

Cytogenetic analysis

Floral buds at the R2 stage, according to the Schneiter and Miller (1981) scale, were harvested in late morning, fixed in Carnoy's solution and maintained at 4°C prior to observation. Chromosome-end arrangements were observed on anther squashes of pollen mother cells (PMCs) at metaphase-I stained with aceto-carmine (Georgieva-Todorova, personal communication). An average of 20 PMCs per plant were scored for chromosome-end arrangements.

Phenotypic and enzymatic markers

Stigmata colour (locus *Stigp*) and CMS *fallax* restoration (locus *RF1-PEF1*) were used as genetic markers because of their monogenic inheritance in the BC1 population. The segregation of six sozyme loci was scored: *Mdh1*, *Mdh2* (malate dehydrogenase), *Pgm1* (phosphoglucomutase), *Sdh1* (shikimate dehydrogenase), *Acp1* (acid phosphatase) and *Me1* (malic enzyme). Isozyme preparations were performed on cotyledons of 8-day- old plants. Electrophoreses were carried out on starch gels in histidine-citrate pH 5.7 buffer (Quillet et al. 1992).

RAPD markers

Total DNA was isolated according to Gentzbittel (1990). Random 10-bp primers were obtained from Bioprobe (France). Amplification reactions were carried out according to Williams et al. (1990) with the following modifications: 0.05 mM of each dNTP, 40 ng of primer, 0.4 units of *Taq* DNA polymerase (Stehelin, France), and 30 ng of genomic DNA. Amplifications were performed in Eppendorf microtubes using either a PHC-3 (Techne, France) or Biometra (Eurogentec, France) thermocycler with an initial period at 94°C for 4 min, followed by 35 cycles at 93°C for 1 min, 38°C for 1 min, and 72°C for 1 min with a final extension cycle at 72°C for 6 min. Amplification products were electrophoresed in 1.6% TBE agarose gels and stained with ethidium bromide. Gels were photographed under transmitted UV light.

Of 102 primers screened, 15 amplified 2–4 fragments unique to the *H. argophyllus* parent. The 133 BC1 progeny were divided into four sets of about 33 individuals. Amplification reactions were performed independently on each set of plants. The reproducibility and the homogeneity of RAPD patterns for the 4 sets of plants were checked by a chi-square test. With the 15 primers, amplifications were homogeneous in the four sets of 38 plants, for 37 out of the 41 polymorphic fragments. The four RAPD fragments which displayed heterogeneous segregation were not used for mapping. Each segregating fragment in the BC1 population was assigned to one locus. The presence of a RAPD fragment among BC1 plants was considered as the heterozygous state and the absence of fragment was assigned to the homozygous state. Data concerning primer sequences and fragment nomenclature are available from the authors.

Hybridisations were performed on Southern transfers of some RAPD gels. RAPD fragments used as probes were purified and reamplified, as previously detailed, plus 1.25 nM of dUTP-digoxigenin (Boehringer, Mannheim) at 50°C for annealing. Hybridisation and detection of digoxigenin hybridised fragments were carried out following the manufacturer's recommendations.

Linkage-map construction and detection of factors affecting pollen viability

Segregation at each locus was checked against the expected 1:1 ratio using a chi-square test. A map was constructed using MAPMAKER version 3.0b (Lander et al. 1987) establishing linkage groups with recombination fraction-values less than 0.35 and a LOD-score threshold of 3.0. Map distances in centiMorgans were calculated using Kosambi's mapping function (Kosambi 1944).

In a first step, loci deviating from the expected 1:1 ratio were not analysed and a basic linkage map was constructed using MAPMAKER. Then, to consider pairs of loci showing segregation distortion,

two models were used in order to estimate the unbiased recombination fraction (r) and the LOD score. Model 1, described by Bailey (1949) and further developed for backcross populations by Lorieux et al. (1995), was used with pairs of loci when the distortion rate observed on one locus is independent (i.e. has not the same biological origin) of the distortion rate displayed by the other locus. In this case, the classical estimator of r is biased, so the corrected estimator of r and the LOD score were re-calculated with the maximum-likelihood method, which takes into account selection rates on each locus. Model 2 was developed because we observed a significant deficit of parental heterozygous individuals in comparison with parental homozygous individuals in some locus-pair segregations. When model 1 or 2 was appropriate, three-point tests taking into account corrections were performed to order distorted loci in linkage groups. In all other cases, two-point and multi-point tests were performed with MAPMAKER.

Associations between genetic markers and pollen viability were detected by analysis of variance with the SAS GLM procedure (SAS Institute Inc. 1987). Marker-loci effects were declared significant when the probability levels associated with F-values were less than 0.01.

Results

Genetic marker segregation and linkage map

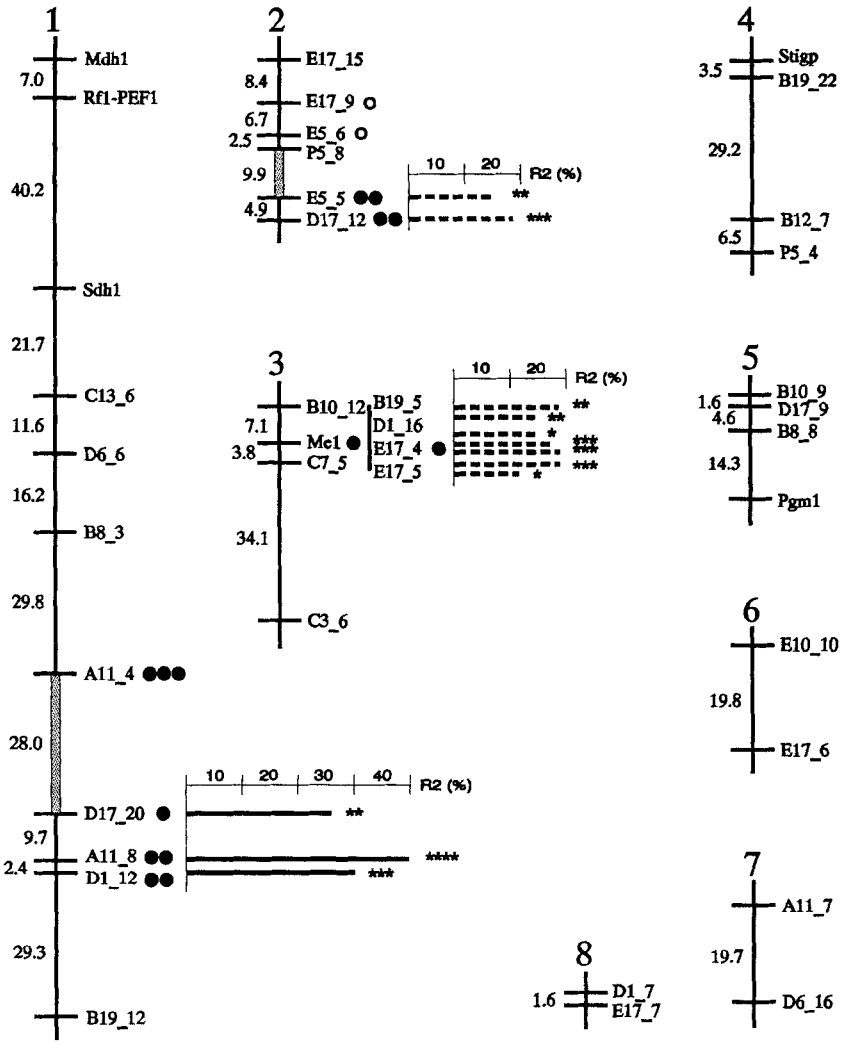
Segregation data for genetic markers were analysed for 133 BC1 progeny. Eleven loci (ten RAPD marker loci and the *Mel* locus) displayed segregation distortion at the 5% level. Eight of these eleven loci showed a deficit, and three an excess, of the enzymatic allele or RAPD fragment from the *H. argophyllus* parent. To eliminate the possibility that segregation distortion was due to poor DNA amplification, gels of three RAPD analyses using the three primers A11, E5 and E17 were Southern transferred and hybridised with one of the corresponding polymorphic fragments from each amplification. Deviation from the expected 1:1 ratio for these fragments, when scored on ethidium bromide-stained gels, was confirmed by the hybridisation data. Moreover, the *E17_5* fragment probe hybridised with four segregating fragments generated with the E17 primer but also with 3 additional fragments ambiguously scored after ethidium bromide staining. Segregation data for these extra fragments were also used for linkage-map construction. A total of 40 RAPD fragment segregations were scored for the mapping of the BC1 population.

The basic map, with 37 non-distorted markers, had eight linkage groups and nine isolated markers with a threshold LOD score of 3 and a maximum recombination fraction of 0.35. Relaxing the mapping condition to include a LOD-score variation between 3 and 2 and a recombination-fraction variation between 0.35 and 0.45, did not place any isolated marker on an existing linkage group. Most of the linked loci were associated with a LOD score greater than 5 and a recombination fraction less than 0.30, except for three intervals on linkage groups 1 and 3. After corrections according to models 1 and 2, the addition of the 11 marker loci showing segregation distortion led to eight linkage groups and nine isolated markers (Fig. 1). Ten of these eleven markers were placed on three linkage groups. Model 1 was applied to correct marker-orders for linkage group 2 because some pairs of linked markers were distorted in opposite orientation. The hypothesis of selection against the parental heterozygous genotypic class (model 2) led to the construction of linkage group 1 with 11 markers covering 196 cM. The final linkage map represented 390 cM with a mean distance between linked markers of 14.4 cM and with 13 of the 31 intervals less than 5 cM. On linkage group 3, seven markers were clustered on approximately 15 cM (Fig. 1).

Genetic markers associated with interspecific pollen viability

The BC1 progeny was produced on PEF1 male-sterile cytoplasm. The *H. argophyllus* no.92 accession restores fertility to this cytoplasm and we observed segregation for this trait in the BC1 population. When restored, plants with PEF1 cytoplasm produced normal quantities of fully viable pollen grains in intraspecific crosses (Serieys and Vincourt 1987). Male-sterile phenotypes have been characterised by a total lack of pollen in the anthers (Laveau et al. 1989). About half of the plants (74 out of the 133 BC1 progeny) used for linkage-map construction, were male-fertile as expected for the segregation of one dominant male-fertility restorer gene. These plants displayed a mean pollen viability of 58.2% ranging between 23.6% and 96.5% (Fig. 2). Parental genotypes of the BC1 population (RHA274 and *H. argophyllus*×RHA274) displayed extreme values for this trait with low intra-genotype variability. The trait distribution in the population was significantly different from normality with at least two modes; therefore, the data were transformed by a logarithm function for further analysis.

One-way analysis of variance revealed significant effects for three markers on linkage group 1. The most important effect was displayed by the *A11_8* locus which explained 39.2% of pollen-viability variation. In order to decrease residual variance and to look for potential marker/trait associations with less important effects, a two-way analysis of variance was carried out with the locus *A11_8* as a fixed factor. Using this procedure two additional genomic regions were detected on linkage groups 2 and 3. The most informative markers, *D17_12* on linkage group 2 and *Mel* on linkage group 3, explained 18% and 18.3% of the total phenotypic variation, respectively. A multiple regression-model analysis, including all the markers with significant effects, explained 82.9% of the total phenotypic variation for pollen viability. For each of these genomic regions, the heterozygous state of markers conferred a negative effect on pollen viability. Moreover, markers strongly linked to pollen viability systematically displayed segregation distortion with a deficit of heterozygous genotypes. In fact, out of the 17 plants homozygous for the loci *A11_8*, *D17_12* and *Mel*, 16 exhibited more than 75% viable pollen (Fig. 3). Only three plants were found heterozygous for these three loci and their pollen viability was less than 30%. The disequilibrum between the number of heterozygous and homozygous plants for these three loci explained in



Unlinked markers: Mdh2, Acp1, B19_10, C3_4, C7_4, D17_6, E10_2, E17_11, C13_5 ooo

Fig. 1 Linkage map based on 48 genetic markers derived from the cross [*H. annuus* cv PEF1-RHA274×(*H. argophyllus* no.92×*H. annuus* cv RHA274)] and location of putative factors controlling pollen viability. Marker-loci names are on the right, and the Kosambi map distances on the left, of the linkage groups *numbered* from 1 to 8. *Grey vertical bars* indicate corrected interval distances in the frame of model 1 for linkage group 2, and significant linkage between loci *A11_4* and *D17_20* in the frame of model 2 for linkage group 1. Segregation distortion with a deficit of the heterozygous genotypes (● $P< 0.05$, ●● $P< 0.01$, ●●● $P< 0.001$) or excess of the heterozygous genotypes (o $P< 0.05$, o o o $P< 0.001$) are indicated on the right of the locus names. Loci listed on the right of linkage group 3 could not be ordered with a likelihood support ≥100:1. *Horizontal lines* indicate the percentage of phenotypic variation (R_2) for pollen viability explained by the individual locus, and *asterisks* represent probability levels (*$P< 0.01$, **$P< 0.001$, ***$P< 0.0001$). *Continuous lines* correspond to the results of a one-way analysis of variance and dashed lines to a two-way analysis of variance with locus *A11_8* as a fixed factor

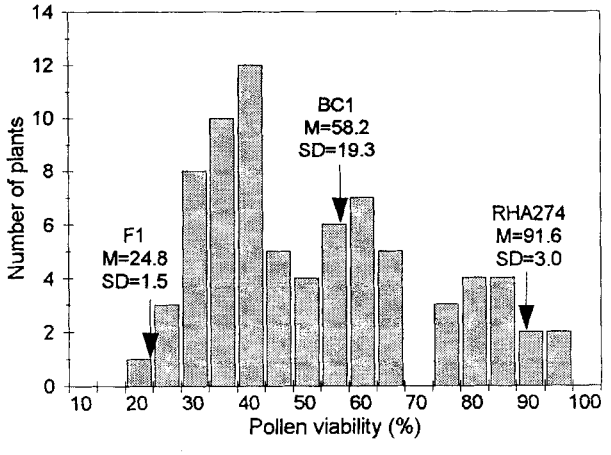


Fig. 2 Pollen-viability distribution for the 74 restored male-fertile BC1 plants. The mean value (*M*) and standard deviation (*SD*) are indicated for BC1 and parental genotypes

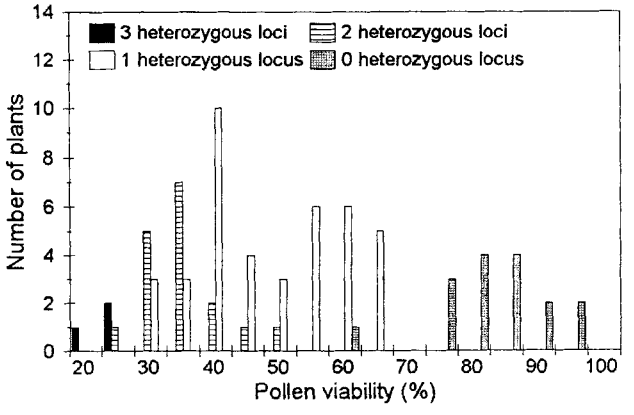


Fig. 3 Pollen-viability distribution for the 74 restored male-fertile BC1 plants according to the homozygous or heterozygous state of the three loci *A11_8*, *D17_12* and *Mel* which display the strongest significant R_2 for pollen viability on linkage groups 1, 2 and 3

Table 1 Meiotic configurations at metaphase-I in *H. annuus*, *H. argophyllus*, *H. argophyllus*×*H. annuus* and BC1 plants. The average value of each plant is followed by the range of variation in all the PMCs

Genotype	Plant	Pollen viability	Number of PMCs scored	Mean number of chromosome pairs involved in			
				Univalents	Rod bivalents	Ring bivalents	Tetravalents
H. annuus (RHA274)	1	93.0	21	0	1.07 (0–3)	15.93 (14–17)	0
H. argophyllus (n. 92)	1	94.9	30	0	1.76 (0–5)	15.24 (12–17)	0
H. argophyllus (n. 92)	1	24.9	21	0.05 (0–1)	6.72 (3–10)	9.85 (7–13)	0.38 (0–4)
× *H. annuus* (RHA274)	2	27.2	32	0	9.0 (4–14)	7.75 (4–13)	0.25 (0–2)
BC1	1	27.2	15	0.07 (0–1)	6.86 (5–10)	9.57 (6–14)	0.50 (0–4)
	2	27.5	31	0.16 (0–1)	7.84 (2–11)	8.94 (5–13)	0.54 (0–4)
	3	31.8	11	0	5.42 (2–7)	11.04 (8–13)	0.54 (0–4)
	4	35.9	13	0	3.23 (2–4)	13.47 (13–15)	0.30 (0–2)
	5	40.0	11	0	2.93 (2–4)	13.48 (12–15)	0.59 (0–2)
	6	54.3	18	0	5.47 (2–10)	11.42 (6–15)	0.11 (0–2)
	7	58.0	25	0.04 (0–1)	6.18 (3–10)	10.22 (7–13)	0.56 (0–2)
	8	62.0	11	0	2.74 (2–4)	14.26 (13–15)	0
	9	70.6	7	0	4.63 (2–6)	12.37 (11–15)	0
	10	74.6	20	0.05 (0–1)	2.55 (1–5)	14.20 (12–15)	0.20 (0–2)
	11	81.8	7	0	3.88 (2–5)	12.84 (12–15)	0.28 (0–2)
	12	83.1	15	0.20 (0–2)	3.18 (1–5)	13.62 (12–15)	0
	13	87.6	23	0.04 (0–1)	2.25 (0–6)	14.71 (11–17)	0
	14	89.7	28	0	3.81 (0–9)	13.19 (8–17)	0
	15	93.0	26	0	2.78 (0–6)	14.22 (11–17)	0

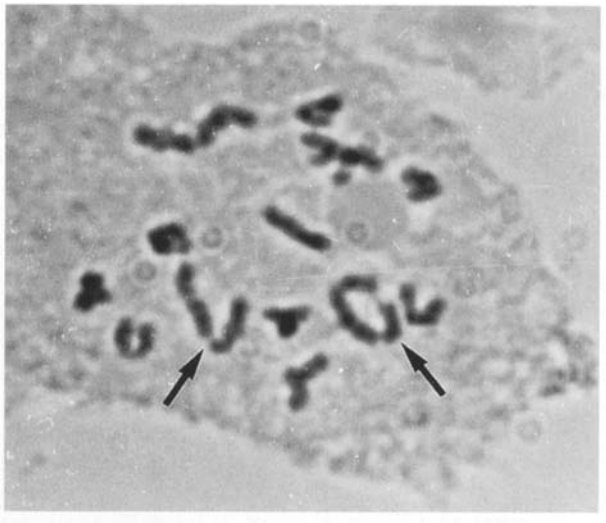


Fig. 4 Chromosome pairing in the interspecific hybrid (*H. argophyllus*×*H. annuus* cv RHA274). Pollen mother cell at metaphase-I showing 13 bivalent and two tetravalent configurations are indicated by *arrows*

part the non-normal distribution observed for this trait. Plants with one or two of these three loci in the heterozygous state had pollen viability ranging from 29 to 62%.

Relation between pollen viability and the meiotic behaviour of F1 and BC1 hybrids

In meiotic preparations, RHA274 and *H. argophyllus* displayed normal chromosome-end arrangements with a majority forming ring bivalents (Table 1). In the F1 hybrids, 5% of PMCs had one tetravalent and less than 1% had two tetravalent (Fig. 4) or univalent configurations. The number of rod bivalents varied from 3 to 14 chromosome pairs in interspecific hybrids, instead of the 0 to 5 found in the parental species. The meiotic behaviour of the 15 BC1 plants was quite variable but the range for the number of rod bivalents, univalents, and the presence of one or two tetravalent configurations, was intermediate between that exhibited by the parental species and the F1 interspecific hybrids. The presence of two tetravalent configurations was observed for the plant with the lowest pollen viability but the relation between pollen viability, and meiotic chromosome pairing was stronger when the mean number of rod bivalents per cell was considered (Fig. 5). In a step-by-step multiple-regression analysis, the mean number of rod bivalents explained 64.5% of the pollen viability, while the mean number of tetravalent configurations explained 6.6%. Two tetravalents and a maximum of 13 ring bivalents were observed for BC1 plants with a pollen viability less than 32%. One tetravalent and a maximum of 15 ring bivalents were displayed by genotypes with a pollen viability between 25% and 83%. When the pollen viability was more than 83%, plants displayed a meiotic behaviour close to the one of parental species, having a maximum of 17 ring bivalents.

Discussion

Genetic map

The unsaturated genetic map has 39 markers assigned to eight linkage groups. In addition there are nine unlinked

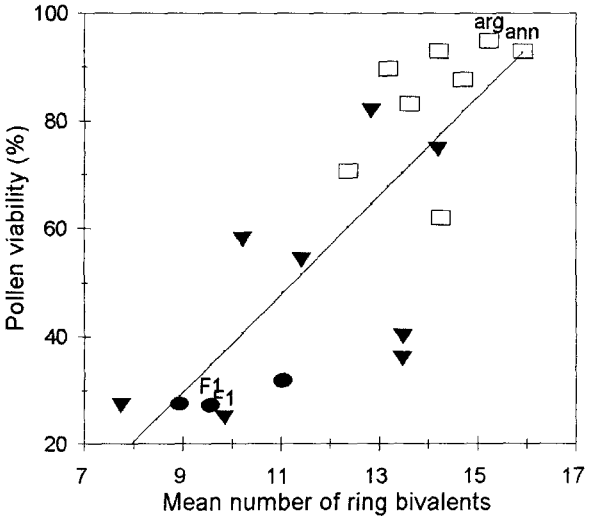


Fig. 5 Correlation between the mean number of ring bivalent per PMC at metaphase-I and the pollen viability of interspecifc BC1 plants, *H. argophyllus*×*H. annuus* cv RHA274 (*F1*), *H. argophyllus* (*arg*) and *H. annuus* cv RHA274 (*ann*). Plants which displayed at least one tetravalent (▼) or two tetravalents (●) among the PMCs scored are indicated

markers. The map covers less than 400 cM and represents a minimum of 20–25% of the genome according to the intraspecific nearly saturated maps for sunflower (Berry et al. 1995; Gentzbittel et al. 1995) or *Helianthus anomalus* (Rieseberg et al. 1993). In our mapping population, the recombination rate was probably reduced because of the genetic divergence between the parental genotypes as in the case of the maize-teosinte maps (Doebley and Stec 1993), interspecific maps in the genus *Lycopersicon* (Miller and Tanksley 1990; Paterson et al. 1988), or in rice interspecific crosses (Causse et al. 1994). Another factor affecting apparent clustering of some RAPD markers may be their non-random location in the genome; however, previous reports have indicated that clustering of RAPD loci, in comparison with RFLP loci was not a general rule (Giese et al. 1994; Kesselli et al. 1992, 1994).

About one-fourth of our markers displayed segregation distortion. The distortion was generally low (only 3 out of the 11 loci showed a significant deviation at $P< 0.001$) but to place some of them on the linkage map required the use of corrected models to estimate unbiased genetic distances and linkage probabilities. Marker positions in the frame of model 1 did not interact with pollen viability. Model 2 was applied when segregation distortion was considered as a consequence of selection against the heterozygous parental genotypic class in locus-pair segregation. This model led to a robust significant linkage between the *A11_4* and *D17_20* loci (LOD score=5.5, r=0.25) and to the construction of linkage group 1 which displayed a disproportional length in comparison to other linkage groups. Linkage group 1 covered 196 cM and represented half of the total map length. This situation could be attributed to a clustering of polymorphic markers in this genomic region because of reduced recombination, non-random distribution, or structural heterozygosity. In this last case, it is possible that the two genomic segments on opposite sides of a translocation break-point exhibit linkage even though they are on different chromosomes in one of the parental species. Such explanations have already been hypothesised for the banana (*Musa acuminata*) linkage map (Fauré et al. 1993).

Genetic factors controlling interspecific pollen viability

The meiotic behaviour of *H. annuus*×*H. argophyllus* hybrids has already been analysed by several authors (Heiser 1951; Kulshreshta and Gupta 1979; Georgieva-Todorova 1984). These different studies have reported the presence of a single tetravalent in a few percent of PMCs. Chandler et al. (1986) made their observation at the diakinesis stage when chiasmata have not yet terminalised; 66% of PMCs with a single tetravalent and 19% of PMCs with two tetravalents were observed. They concluded that *H. annuus* and *H. argophyllus* differed by two reciprocal translocations.

Our results are consistent with the data of Chandler et al. (1986); however our observations were made at the metaphase-I stage when chiasmata were terminalised and therefore ring tetravalent configurations were not as evident. We observed a high number of rod bivalents in hybrid meiosis whereas few were observed in the parental species. Partial pairing in F1 interspecific hybrids might be due to early partition of two tetravalents. The abnormalities in the meiotic behaviour of BC1 plants were correlated with a reduction in pollen viability. These results support the hypothesis that chromosome rearrangements are the preponderant factors for the observed reduction in pollen viability in the progeny.

Using the genetic map, three genomic regions were detected which, in combination, explain about 80% of the pollen-viability variation in the BC1 population. A genetic factor having a major effect on pollen viability is located close to the *A11_8* locus on linkage group 1. This location is consistent with the hypothesis of structural heterozygosity explaining the abnormal length of this group and the decrease in pollen viability due to reciprocal exchanges. Markers associated with pollen viability on linkage groups 2 and 3 displayed less effects. Although these linkage groups did not have a particular structure, we suggest that these genomic regions are also located on opposite sides of a second translocation break-point because of the strong relation between pollen viability and chromosome-end rearrangements. The locations of translocation break-points have been reported in intraspecific crosses of rye (Figueras et al. 1985), or in interspecific crosses of *Brassica* (Kianian and Quiros 1992), by comparison of marker-loci positions among different crosses. In a *Lens* interspecific cross, Tadmor et al. (1987) used the association of pollen viability, meiotic configurations, and isozyme loci to locate a translocation break-point.

Theoretically, segregation for the four chromosome pairs involving two independent reciprocal translocations produce 2/16 of parental equilibrated gametes and 2/16 of recombinant equilibrated gametes. Equilibrated gametes display entire chromosomes of one or the other parent species. Other gametes (12/16) have unbalanced chromosome compositions which are likely to be lethal (Grant 1971). Theoretical interspecific hybrid pollen should have a maximum viability of 25% when unbalanced gametes are totally lethal. Our experimental data on F1 pollen viability approximate this value (Fig. 2); however, the three genomic regions associated with pollen viability displayed segregation distortion with a systematic deficit in heterozygous genotypes. If chromosome-end rearrangements were the only cause of reduction in pollen viability, the genome structure of each parental species should be equally represented in the viable gametes and we would not expect any gametic selection in the meiosis of the hybrid. Thus, we suggest that the segregation distortions observed for genomic regions linked to pollen viability were of post-meiotic origin. The deficit in heterozygous genotypes can be explained by two hypotheses which remain to be tested: (1) distortions are due to pairing abnormalities which lead to an abortion of heterozygous embryos (Stebbins 1971), or (2) distortions are due to genes associated with interspecific compatibility and these are located close to the genomic regions involved in the expression of pollen viability. Germination and/or tube growth of pollen grains with the *H. argophyllus* genome structure could be prevented on the stigmata of the recurrent parent, *H. annuus*, in this backcross population. Mechanisms which lead to selective fertilization have been investigated in another interspecific cross between *Helianthus* sect. *Helianthus* species. Rieseberg et al. (1995) have reported pollen-competition experiments between *H. annuus* and *H. petiolaris*. In the absence of pollen competition, they observed a high percentage of filled achenes whatever the pollinator and receptor parent species. This suggests that interspecific embryos are fully viable. In using pollen mixtures of both species, the intraspecific pollen gave the higher number of filled achenes, independently of the ratio of interspecific pollen and of the species used as pollen receptor. However, they did not observe differences for pollen-tube growth rates of the two species. They concluded that selective fertilisation, involving complex mechanisms, seems to play an important role in preventing the formation of interspecific hybrids.

In using interspecific crosses as genetic resources for the improvement of sunflower, recombination between the sunflower and wild species genomes during several mating cycles is the preliminary step for introgressing useful agronomic traits. Our results indicate that genetic markers linked to pollen viability could be used to prevent the elimination of some agronomicallly important genotypes because of their low fertility and the segregation distortion probably associated with structural heterozygosity.

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