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Occurrence of partial hybrids in wide crosses between sunflower (*Helianthus annuus*) and perennial species *H. mollis* and *H. orgyalis*

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Abstract Hybridisation between the annual diploid sunflower (*Helianthus annuus)* and the perennial diploid species *Helianthus mollis* and *Helianthus orgyalis* was obtained by means of a normal crossing procedure or embryo rescue. Hybridisation success was low. All plants examined cytologically appeared to be diploid. However, the phenotypes of these diploids were not intermediate between the parents and, despite great variation, they resembled the female parent-type predominantly. Thirty five percent of plants issued from sunflower pollinated with perennial *Helianthus* had a phenotype resembling the female sunflower parent. On average, only 5% of the minimum number of expected RAPD and RFLP bands from male parents were recovered in plants produced from mature seeds after pollination of sunflower by *H. mollis.* More hybrids were found among plants obtained from embryo rescue, with an average of 25% of the male parent bands recovered per plant. Analysis of individual plants indicated the occurrence of various levels of hybridisation. There was a significant positive correlation between the number of phenotype traits related to hybrid status and the number of bands derived from the male parent. A single hybrid plant might possibly represent the product of a 'normal' hybridisation event. The mechanisms behind these unusual events and the consequences for the breeder are discussed.

Keywords *Helianthus* · Sunflower · Interspecific hybrid

Introduction

Sunflower (*Helianthus annuus* L.) is an important oil crop with a narrow genetic background. Introgressed plants from wild forms and foreign species are used successfully by breeders (Korell et al. 1996a, b). Wide crosses between different species of *Helianthus*, especially between annual and perennial species, are difficult to carry out, and successful hybridisation is usually obtained through the use of in vitro embryo rescue methods (Korell et al. 1996a). In this genus, all annual species and the majority of perennial species have the same number of chromosomes (2n=34). However, their genomes are different. We characterised at least three different genomes in addition to the common C-genome background in the genus through a diversity study using RAPDs. Sunflower and all annual species carry the two genomes H and C and belong to the section *Helianthus. Helianthus mollis* Lam., *Helianthus orgyalis* DC and many perennial species carry the C, P and A genomes, and belong to the section *Atrorubentes*. Other perennial species carry only the C and P genomes, and belong to the *Ciliares* section (Sossey-Alaoui et al. 1998). *Helianthus* appears as a segmental allopolyploid or palaeopolyploid genus (Wendel 2000). This genomic classification agrees with the classification of Schilling and Heiser (1981) based on phenotype.

Attempts to obtain wide interspecific hybridisation in the genus *Helianthus* have met with some success, especially with the help of embryo-rescue techniques (Georgieva-Todorova 1984; Christov 1991; Jan 1996; Sukno et al. 1998). Kraüter et al. (1991) obtained numerous hybrids between sunflower and perennial *Helianthus* via embryo rescue. Unexpectedly, the pollen viability of some hybrids was high. In annual×perennial crosses, it appeared that parental chromosomes were present as additions, and that amphiploidisation was necessary for good-fertility levels to be recovered. Natali et al. (1998) have reported on crosses between *H. annuus* and Jerusalem artichoke (*Helianthus tuberosus*) using somewhat different methods and techniques. Their chromosome studies suggest the addition of parental haploid numbers. However, these hybrids were found to be genetically polymorphic, with DNA alterations. Similarly, extensive genome changes were observed in newly synthesised

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Brassica allopolyploids (Song et al. 1995). In this study of reciprocal allopolyploids, cytoplasmic effects could not be excluded. However, their preliminary data suggested that chromosome rearrangements related to aberrant meiosis could be a major factor contributing to genome change. These rearrangements, if subject to meiosis in *Brassica* hybrid plants, are possibly very different from the results of hybridisation between barley and *Hordeum bulbosum* (Kasha and Kao 1970). In the latter case, genome rearrangements or haploidisation are observed as a direct consequence of alien pollination, and may again be pre-meiotic events. The results discussed by Natali et al. (1998) were also obtained prior to meiosis in hybrid *Helianthus* plants.

We made wide crosses (Cazaux et al. 1996; Faure et al. 2000) between sunflower (*H. annuus* L.) and the wild perennial species *H. mollis* Lam. (Heiser et al. 1969) and *Helianthus orgyalis* DC as defined by Watson (1929). We found that results concerning phenotypic and molecular markers were difficult to interpret, with much variation among the hybrids obtained from the same experimental cross. Moreover, the hybrids showed a closer resemblance to the female parent than to the male parent. In the present paper, this work is reconsidered and continued with the view of estimating the effects of hybridisation between sunflower and perennial species of *Helianthus* on genotype and phenotype in the different hybrids. A preliminary model for further investigation is proposed.

Materials and methods

A total of 15 crosses were studied: (1) sunflower×*H. mollis* between female sunflower and male *H. mollis* (crosses 1 to 12) and the reciprocal in crosses 14 and 15, and (2) female sunflower×male *H. orgyalis* (cross 13). All hybrid seedlings and plants were grown in a greenhouse with a controlled photoperiod (16-h day) and thermoperiod (day 25°C, night 18°C).

Genotypes

Sunflower: different Pet1 cytoplasmic male-sterile cultivated lines 85A3, FT2603, Ha89 and F1 Ha89*AA724 were pollinated with perennial *Helianthus* species. Perennial *Helianthus* species were pollinated with the male-fertile sunflower lines LA, WG, Ha89 and Rha274.

Perennial *Helianthus*: different clones of *H. mollis* accessions HM 230 (from PI435749), HM 600 and HM742 (both from PI 468761) were used as male parents. Accession HO 108 from VIR (Russia) was also used as a male parent and was classified as *H. orgyalis* as defined by Watson (1929). Different clones of *H. mollis* accessions HM 742 (from PI 468761) and accession HM 286 (derived from VIR-Russia) were used as the female parent. HM 286 segregates for male sterility.

Crossing techniques

Pollination of sunflower by perennial Helianthus species

A total of 13 interspecific crosses were performed in the field during the summer of 1994 (crosses 1 to 11) and the summer of 1997 (crosses 12 and 13).

In 1994, mature hybrid seeds were recovered from crosses 1 to 11and plated in Petri dishes before planting in a greenhouse. In 1997, 7-day old hybrid embryos of crosses 12 and 13 were transplanted in vitro on MS-modified growth medium (Alissa et al. 1986) in an attempt to rescue embryos.

Pollination of perennial H. mollis *by sunflower*

Crosses between two *H. mollis* accessions, one self-incompatible (HM 742) and one male-sterile (HM 286) as female parents and sunflower as the male parent, were carried out in 1994. Mature hybrid seeds of crosses 14 and 15 were recovered and plated in Petri dishes before planting in the greenhouse in the same conditions as those of other crosses.

DNA analysis and cytological observations

Crosses 1 to 11 with *H. mollis* as the male parent and crosses 14 and 15 with *H. mollis* as the female parent were characterised by RAPD bands specific to the perennial species, as described by Sossey-Alaoui et al. (1998, 1999). Primers A5, A12, A15, A16, A19, B19, C2, C4, C12, C14, C15, C16, C19, E3, E9 and E15 (Operon) were used.

Cross 12 with *H. mollis* as the male parent and cross 13 with *H. orgyalis* as the male parent were characterised in 1997–1998 using RFLP fragments. DNA preparation, DNA restriction with *Eco*RI and *Hind*III, and Southern blots, were made according to the methods described in Lacombe et al. (1999). The probes used were sunflower cDNA of Δ9, Δ12 desaturase from Kabbaj et al. (1996) and Lacombe et al. (1999), respectively, Δ15 desaturase and Tip from Sarda et al. (1997), Sdi-6, Sdi-8, Sdi-9 and Sdi-10 from Ouvrard et al. (1996). Chromosome counts were performed on root-tips of crosses 1 to 11, following the technique of Bervillé et al. (1993).

Phenotype

The following characteristics were recorded on hybrid plants: plant height (cm), stem branching, presence of anthocyanin colour, days from planting to flowering, restoration of Pet1 cytoplasmic male sterility (sex), alterations of male organs (normal stamen size or reduced size), pollen viability (as measured by the method of Alexander's 1969), and seed number after selfing or back-crossing to sunflower (hybrid plants were selfed or back-crossed to sunflower depending on their male fertility status).

Statistical analysis

In order to detect an eventual structure in the tables of qualitative results, correspondence analyses (Benzécri 1973; Greenacre 1984; Goodman 1988; SAS CORRESP procedure 1992) of the qualitative phenotype observations were carried out, and contingency tables produced of RAPD bands for all hybrid plants from mature seed of sunflower pollinated by *H. mollis* (crosses 1 to 11).

Results

Crosses of sunflower pollinated by perennial *Helianthus*

Hybridisation success

From a total of 293 sunflower heads pollinated by *H. mollis* 0.87 hybrid plants were recovered on average per pollinated head after in vitro embryo rescue. From 42 inflorescences pollinated by *H. orgyalis*, average hybrid plant recovery after in vitro embryo rescue was 0.95, and

Table 1 Phenotype and RAPD of progeny from from sunflower pollinated by *H. mollis* (crosses 1 to 11) produced from mature seed. NB: * indicates that the trait differs significantly ($P<0.05$) from sunflower type plants. Stem branching: B for branched, N for non-branched. Sex: F for female, M for hermaphrodite. Stamen size: S for small stamens, N for normal size

Code	Cross	Pedigree	Presence of Antho-cyanin	Stem branching	Days to flowering	Sex (CMS restorer)	Stamen size	Pollen viability (%)	Head size (cm)	Number of self seeds	Number of back–cross seeds	Hybrid traits	Intro-gressed bands	Expected number of bands from male in F_1
AM1	1	S×HM230–7		N	61	F			5.25*		123	1	1	24–48
AM2	1	S×HM230–7		B*	58	F			5.75*		162	2	4	24–48
AM3	1	S×HM230–7		N	56	F			9		293	0	0	24–48
AM4	1	S×HM230–7		B*	61	M*	N	97.7	8	282		2	3	24–48
AM8	1	S×HM230–7		N	57	F			9.75		321	0	0	24–48
AM12	1	S×HM230–7		N	63	F			7.75		189	0	0	24–48
AM14	2	S×HM230–5	A*	N	56	F			8.5		415	1	0	30–60
AM15	2	S×HM230–5		N	58	F			10.5		491	0	0	30–60
AM16	2	S×HM230–5	A*	B*	63	M*	N	92.3	5.5*	0*		5	2	30–60
AM17	2	S×HM230–5		N	60	M*	N	98.6	8.25	334		1	0	30–60
AM18	2	S×HM230–5	A*	B*	58	F			8		149	2	3	30–60
AM19	2	S×HM230–5		B*	58	M*	N	93.7	9.25	311		2	2	30–60
AM20	2	S×HM230–5		N	56	F			9.5		337	0	2	30–60
AM21	2	S×HM230–5		B*	72*	M*	S*	94.2	4.5*	1*		6	3	30–60
AM22	2	S×HM230–5		N	58	M*	N	100	8.5	219		1	1	30–60
AM23	2	S×HM230–5		B*	61	M*	S*	99.3	9.25	21*		4	1	30–60
AM24	2	S×HM230–5		N	56	F			7.5		295	0	1	30–60
AM25	2	S×HM230–5	A*	B*	55	F			4.85*		20*	4	5	30–60
AM26	2	S×HM230–5	A*	B*	50*	F			4.25*		40*	5	7	30–60
AM30	3	S×HM742–5		N	59	F			7.75		211	0	0	20–40
AM31	3	S×HM742–5		N	57	F			6.5		317	0	0	20–40
AM32	3	S×HM742–5		N	59	M*	N	99.3	9.5	240		1	0	20–40
AM33	3	S×HM742–5		N	57	F			8		271	0	0	20–40
AM34	3	S×HM742–5	A*	N	63	F			3.75*		4*	3	4	20–40
AM35	4	S×HM742–3	A*	B*	63	F			3.75*		14*	4	2	28–57
AM37	4	S×HM742–3		N	73*	M*	N	97.0	8.5	185		2	4	28–57
AM38	4	S×HM742–3	A*	B*	57	M*	N	4.2*	1.25*	0*		6	3	28–57
AM40	5	S×HM230–10		N	58	F			8.5		307	0	1	16–32
AM41	5	S×HM230–10		N	63	F			7.75		142	0	0	16–32
AM42	5	S×HM230–10		N	55	F			9.5		378	0	1	16–32
AM43	5	S×HM230–10		N	63	F			7.75		309	0	1	16–32
AM44	6	S×HM600–10		N	59	F			8.5		288	0	1	19–39
AM46	6	S×HM600–10		N	58	F			9.25		313	0	1	19–39
AM47	6	S×HM600–10		N	63	M*	N	98.8	9.5	246		1	1	19–39
AM48	6	S×HM600–10		N	57	M*	N	98.8	9.5	250		1	1	19–39
AM55	7	S×HM742–1		N	64	F			7		149	0	2	22–45
AM56	7	S×HM742–1		N	60	F			7.75		220	0	0	22–45
AM57	7	S×HM742–1		N	62	F			8		310	0	0	22–45
AM58	7	S×HM742–1		B*	82*	M*	S*	22*	4.5*	0*		7	1	22–45
AM59	7	S×HM742–1		N	60	F			8.5		304	0	0	22–45
AM60	7	S×HM742–1		N	64	F			7.75		190	0	0	22–45
AM66	8	S×HM230–7		N	73*	F			7.75		18*	2	5	24–48
AM71	9	S×HM230–5		N	58	M*	N	93	9.5	297	407	1	1	30–60

Table 1 (continued)

Code	Cross	Pedigree	Presence of Antho-cyanin	Stem branching	Days to flowering	Sex (CMS restorer)	Stamen size	Pollen viability (%)	Head size (cm)	Number of self seeds	Number of back–cross seeds	Hybrid traits	Intro-gressed bands	Expected number of bands from male in F_1
AM72	9	S×HM230–5		N	56	F			7.5		236	0	1	30–60
AM73	9	S×HM230–5		B*	61	F			9		227	1	0	30–60
AM74	9	S×HM230–5		B*	63	F			7.75		151	1	0	30–60
AM75	9	S×HM230–5		B*	76*	F			4.75*		10*	4	0	30–60
AM76	9	S×HM230–5		N	67*	M*	N	21*	9.5	3*		4	1	30–60
AM77	9	S×HM230–5		N	63	M*	N	100	8.5	253		1	0	30–60
AM78	10	S×HM742–1		N	61	M*	N	98	9	165		1	2	22–45
AM79	10	S×HM742–1		N	63	F			10.5		322	0	0	22–45
AM80	10	S×HM742–1		N	65	F			8.75		304	0	0	22–45
AM81	10	S×HM742–1		N	70*	M*	S*	98	11	352		3	0	22–45
AM82	10	S×HM742–1		N	59	F			9.75		296	0	0	22–45
AM83	10	S×HM742–1		N	70*	M*	S*	76	10.75	11*		4	1	22–45
AM84	10	S×HM742–1		B*	66	F			8.75		175	1	1	22–45
AM85	10	S×HM742–1		N	70*	M*	S*	98	6.75	0*		4	0	22–45
AM86	11	S×HM230–7		N	70*	F			10.5		53*	2	0	24–48
AM87	11	S×HM230–7		N	70*	F			10.75		105	1	0	24–48
AM88	11	S×HM230–7		N	62	F			11.25		72*	1	0	24–48
AM90	11	S×HM230–7		B*	60	F			9.25		214	1	0	24–48
AM92	11	S×HM230–7		B*	59	F			11.25		186	1	0	24–48

the range for both experiments was between 0 and 26 embryos recovered per head. When embryo culture was not used, mature viable seed recovery was lower.

Phenotype analysis

Twenty nine plants out of 84 studied were similar to the parent female sunflower for all traits observed. These plants were noted "0" for hybrid traits in Tables 1 and 2. Plants with quantitative traits marked with an asterisk were significantly different from the phenotype of the female sunflower (Student *t* test, $P<0.05$), after grouping the crosses of experiments 1 to 11 (Table 1) and crosses 12–13 (Table 2) by year. Qualitative traits noted with an asterisk indicate unambiguously a wild or hybrid status (sex, occurrence of small stamens, presence of stem branching, and presence of anthocyanin).

Hybrids with *H. mollis.* Phenotypes of hybrids between Pet1 cytoplasmic female sunflower and *H. mollis* resembled cultivated sunflower, or showed intermediate traits (Tables 1 and 2). Only 46 plants out of 71 manifested dominant *H. mollis* traits or traits related to interspecific hybrid sterility. Compared with the parental sunflower phenotype, 25 plants had branched stems, 18 plants were late flowering, one early and 25 male fertile. No pattern was detected using correspondence analysis (SAS 1992) of the qualitative phenotype observations on all hybrid plants that originated from mature seed of sunflower pollinated by *H. mollis* (crosses 1 to 11).

Hybrids with *H. orgyalis.* Phenotypes of hybrids between Pet1 cytoplasmic female sunflower and *H. orgyalis* were similar to sunflower or were intermediate. Only 9 plants out of 13 manifested dominant *H. orgyalis* traits or traits related to interspecific hybrid sterility (Table 2). Compared with the sunflower phenotypes: one plant had branched stems, one plant was late flowering and one early, eight were male-fertile, and one was male-fertile with reduced pollen viability and low self-fertility.

Taking these results together, from a total of 84 plants (Tables 1 and 2) only 55 manifested dominant *H. mollis* or *H. orgyalis* traits or traits related to interspecific hybrid sterility. When compared with the female sunflower parent, 19 plants were late flowering, two were very early, 26 had branched stems and 33 plants were male-fertile; 15 male–fertile plants had small stamens or reduced pollen viability (<76%) or low self fertility (<22 seeds by head); 12 male-sterile plants had low cross fertility after pollination by sunflower (<73 seeds by head).

DNA analysis

Cytological examination of 20 hybrid plants produced from mature seeds of sunflower pollinated by *H. mollis* (crosses 1 to 11) indicated diploidy ($2n=34$). Analysis of all crosses in which sunflower was pollinated by *H. mollis*, indicated that all the RAPD and RFLP bands of the female parent were found in the hybrids. In con-

Table 2 Phenotype and RFLP of progeny from sunflower pollinated by *H. mollis* or *H. orgyalis* (crosses 12 and 13) produced by means of embryo rescue. NB: *indicates that the trait differs significantly from sunflower type plants ($P<0.05$). Stem branching: B for branched, N for non-branched. Sex: F for female, M for hermaphrodite. Stamen size: S for small stamens, N for normal size. MD: missing data

Code	Cross	Pedigree	Plant Height (cm)	Stem branching	Days to flowering	Sex	Stamen size	Pollen viability (%)	Number of Self seeds	Number of back-cross seeds	Number of Hybrid traits	Number of foreign RFLP	Expected number of RFLP from male in F_1
R71	12	S×HM 230 –11	165	N	50	F				570	0	0	22–45
R72	12	S×HM 230 –11	145	N	56	F				69*	1	3	22–45
R73	12	S×HM 230 –11	164	B*	98*	M*	N	0*		6*	5	3	22–45
R74	12	S×HM 230 –11	145	B*	84*	F				0*	3	6	22–45
R75	12	S×HM 230 –11	200*	B*	78*	M*	N	3*		5*	6	10	22–45
R76	12	S×HM 230 –11	155	B*	86*	M*	S*	6*		2*	6	11	22–45
R77	12	S×HM 230 –11	54*	B*	88*	M*	S*	4*		1*	7	9	22–45
R78	12	S×HM 230 –11	104	B*	73*	M*	N	6*		8*	5	10	22–45
R79	12	S×HM 230 –11	150	B*	95*	F				7*	3	7	22–45
R80	13	S×HO 108	115	N	50	F				508	0	0	28–57
R81	13	S×HO 108	125	N	48	F				167	0	0	28–57
R82	13	S×HO 108	138	N	54	M*	N	99	483		1	3	28–57
R83	13	S×HO 108	125	N	50	M*	N	98	125		1	13	28–57
R84	13	S×HO 108	115	N	49	M*	N	98	439		1	5	28–57
R85	13	S×HO 108	120	N	50	M*	N	96	239		1	4	28–57
R86	13	S×HO 108	120	N	55	M*	N	99	MD		1	7	28–57
R87	13	S×HO 108	145	N	59	F				517	0	0	28–57
R88	13	S×HO 108	120	N	62	M*	N	97	148		1	5	28–57
R89	13	S×HO 108	135	N	56	F				380	0	1	28–57
R90	13	S×HO 108	195*	B*	83*	M*	S*	35*		18*	7	37	28–57
R91	13	S×HO 108	120	N	68	F				278	1	1	28–57
R92	13	S×HO 108	115	N	39*	M*	N	98	521		2	8	28–57

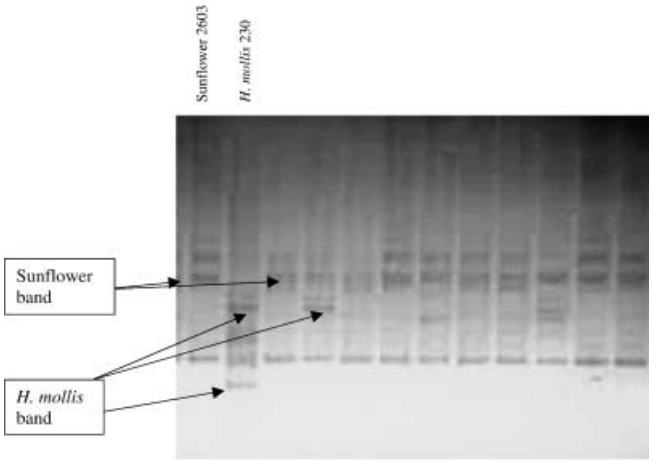

Fig. 1 RAPD bands of progeny from sunflower pollinated by *H. mollis* produced from mature seed (C16 primer)

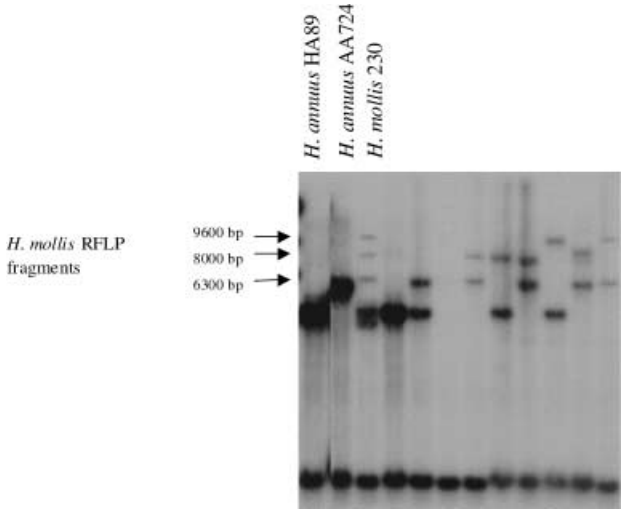

Fig. 2 RFLP autoradiogram of progeny from sunflower pollinated by *H. mollis* produced by means of embryo rescue. DNA was restricted by *Eco*RI and hybridised with the *Sdi-6* probe

Table 3 Synthesis of results of sunflower pollinated by *H. mollis* and by *H. orgyalis*.

A. Pollination by *H. mollis.* Mature seeds (1994). Correlation between occurrence of hybrid traits and bands: +0.49 p=0.000

Item	Number of plants	Mean frequency of hybrid traits	Mean frequency of introgressed RAPD bands per plant	Minimum expected number of *H. mollis* bands in complete hybrids
Hybrid phenotype	38	2.47	1.55	26.1
Sunflower phenotype	24	0	0.46	22.3
Total	62	1.52	1.13	24.6
Range		0 to 7	0 to 7	16 to 30

B. Pollination by *H. mollis* and *H.orgyalis.* Embryo rescue (1997). Correlation between hybrid trait occurrence and fragment occurrence: +0.67 *P*=0.001

Item	Number of plants	Mean frequency of hybrid traits	Mean frequency of introgressed RFLP fragments per plant	Minimum expected male fragments in "true" hybrids
Hybrid phenotype	17	3.06	8.35	25.2
Sunflower phenotype	5	0	0.20	26.8
Total	22	2.36	6.50	25.6
Range		0 to 7	0 to 37	22 to 28

trast, only a minority of RAPD bands of the wild male parent was found in these progenies (Tables 1 and 2, Fig. 1). The synthesis in Table 3 indicates that, of the plants obtained from hybridisation with *H. mollis* without embryo rescue, an average of only 1.1 RAPD bands were introgressed compared to an expected range of between 24.6 and 49.2 bands in true hybrids. The minimal assumption is that every band indicates a single copy of the corresponding DNA (heterozygosity) in *H. mollis* (24.6 bands expected), and the maximal assumption is that every band from the wild species indicates two copies of the DNA (homozygosity) in the *H. mollis* parent (49.2 bands). An average of 6.5 introgressed RFLP fragments were found in embryo-rescued plants obtained by hybridising sunflower with *H. mollis* and *H. orgyalis*, compared with a minimum expected of 25.6 and a maximum of 51.2 (Table 3, Fig. 2). Only plant R90 (Table 2) might be the result of classical hybridisation, with 37 foreign RFLP fragments (which falls within the range of the expected 28 to 57 RFLP fragments).

Analysis of RAPDs and RFLPs suggested that only 41 plants out of 71 originating from pollination by *H. mollis* had introgressed fragments; eight plants out of the 13 obtained after pollination with *H. orgyalis* had introgressed fragments.

Novel RFLPs, undetected in the parents, appeared with Sdi-6 and Sdi-10 probes in 12 hybrid plants out of the 22 studied.

Correspondence analysis (SAS 1992) did not detect any pattern in the occurrence of introgressed fragments in the different hybrids produced from mature seed of sunflower pollinated by *H. mollis* (crosses 1 to 11).

Table 4 Phenotype and RAPD of progeny from the pollination of *H. mollis* by sunflower and developed from mature seed

Pedigree	Cross	Number of plants	Complete stem branching %	Sessile leaf %	Pollen viability of male fertile %	Seed set in selfed male fertile	Seed set after backcross	Plants lacking *H. mollis* bands	Plants with sunflower bands	Range of sunflower bands per plant	Average expected no. of *sunflower* bands
HM 286×S	14	17	100	100	77.5	<0.1	<0.6	0	16	0 to 6	28.8
HM 742×S	15	5	100	100	78.7	0	<0.4	0	4	0 to 2	31

However, some associations between fragments and hybrid plants were observed:

(1) Four RAPD bands derived from *H. mollis* (A16_400, A19_300, C12_550, C12_1300) were found in a single plant exclusively (AM25),
(2) Two RAPD bands derived from *H. mollis* (A16_1050 and E3_475) were found in plant AM78 exclusively.

Relationships between phenotypes and fragments

The average number of RAPD and RFLP bands from the male parent found among the plants with hybrid phenotypes was compared with the results for plants with the sunflower phenotype. Plants with hybrid phenotypes had outstandingly superior numbers of introgressed or recombined bands in both types of interspecific cross: 1.55 bands on average, compared to 0.46 band for hybrids from mature seeds of sunflower pollinated by *H. mollis* (crosses 1 to 11, Table 3), and 8.35 bands compared to 0.20 for hybrids from the embryo rescue of sunflower pollinated by perennial *Helianthus* (crosses 12 and 13, Table 3). However, the mean number of introgressed bands from the wild parent found in these hybrid plants, 1.55, is still lower than expected: a minimum of 26.08 for hybrids produced from mature seeds of sunflower pollinated by *H. mollis* (crosses 1 to 11), and 8.35 compared to a minimum of 25.18 in hybrids obtained from the embryo rescue of sunflower pollinated by perennial *Helianthus* (crosses 12 and 13). A highly significant correlation coefficient of +0.49 (p=0.000) was found between the occurrence of introgressed bands and hybrid traits, in hybrids originating from mature seeds of sunflower pollinated by *H. mollis* (crosses 1 to 11, Table 3), and a similar correlation of +0.67 (p=0.001) in hybrids obtained from the embryo rescue of sunflower pollinated by perennial *Helianthus* (crosses 12 and 13, Table 3).

Comparison of plants from mature achenes and from embryo rescue

Crosses 1 to 11 were obtained from mature seed, harvested in 1994. Crosses 12 and 13 were obtained using embryo rescue in 1997. The mean number of introgressed RAPD bands for crosses 1 to 11 taken as a whole is 1.13 bands per plant and 1.52 hybrid traits, compared with 6.5 introgressed RFLP fragments and 2.36 hybrid traits for crosses 12 and 13 (Table 3). The minimum expected introgressed DNA characters are comparable: 24.6 for crosses 1 to 11 and 25.5 for crosses 12 and 13 (Table 3).

Crosses of *H. mollis* pollinated by sunflower

Hybridisation success

Out of a total of 403 inflorescences of *H. mollis* pollinated by sunflower, we obtained on average only 2.07 viable hybrid plants per head after in vitro embryo rescue, with a range between 0 and 32 of viable embryos per inflorescence.

Phenotype

The results obtained for 22 hybrid plants are presented in Table 4. All hybrid plants had branched stems and sessile leaves, typical of the wild female parent. Some male-sterile hybrids without anthers were obtained, possibly related to the male-sterile status of the *H. mollis*-accession HM 286 parent plants used. All plants with developed anthers had viable pollen (over 75% viable). Seed set after selfing or back-crossing to sunflower was very low, less than one seed per head.

DNA analysis

All *H. mollis* bands were observed in all the hybrids analysed. Only 0 to 6 *H. annuus* fragments were found in these hybrid plants, while a range from 26 to 31 *H. annuus* bands was expected on the basis of RAPD analysis of the parent lines (Table 4).

Discussion

It is striking that the progeny produced from interspecific crosses did not manifest the expected ratios of a true F_1 hybrid, neither when sunflower was the female parent, nor when the wild species was the female. The descendants appear as non-Mendelian partial hybrids. The amount of introgression in these partial hybrids appears quantitatively stochastic, with much variation among de-

scendants within a cross and much variation between different crosses. The only apparent pattern that could be discerned was related to the observed transfer of four bands in two specific hybrid plants, and only in these plants. This could either imply that these grouped markers are redundant, or that a larger fragment was transferred.

Further, we observed more wild and hybrid sterility traits, as well as introgressed markers, among hybrids derived from embryo rescue (crosses 12 and 13) compared to hybrids grown from mature seed (crosses 1 to 11). This could indicate that there is a relationship between the mode of hybrid recovery and the genotype recovered.

The same tendencies were observed in all crosses: the phenotypes were predominantly similar to the female parent, with striking differences between reciprocal crosses (Table 4). In parallel, RAPDs or RFLP fragments from the female parent were conserved and fragments from the male parent were much less-frequent.

Non-parental RFLPs appeared with two probes in hybrid plants prior to meiosis in different hybrids produced from the embryo rescue of sunflower pollinated by perennial *Helianthus* (crosses 12 and 13). This unusual observation can be compared with the results of Natali et al. (1998) obtained from crosses between *H. annuus* and Jerusalem artichoke (*H. tuberosus*). They observed an alteration in heterochromatin condensation and DNA content in first-generation hybrids. They also found that highly repetitive DNA probes produced restriction patterns for the hybrids that differed from those of the parents They interpreted these phenomena as responses to genomic shock following the interspecific cross (McClintock 1984). In the face of such complex results, and given the huge variation between hybrid plants, we believe that the observed results cannot be explained using a single simple model. Two general models are proposed here.

(1) Complete Mendelian hybrids. These are possibly more frequent after embryo rescue, and the R90 plant (Table 2) may represent one. In this model, the addition of parental genomes is generally observed after hybridisation, followed by chromosome doubling, e.g. with colchicine treatment leading to the production of amphiploids (necessary for the recovery of fertile genotypes). Evidence supporting this model has been obtained in many wide crosses, and in *Helianthus,* on the basis of the results of Jan et al. (1996), and Sukno et al. (1998).
(2) Partial hybrids. They are more frequent after normal seed maturation, possibly because classical Mendelian interspecific hybrids are naturally screened out. Unexpectedly, the partial hybrids recovered appear to be diploid. The steps involved in this process are hypothesised as:

(i) Early elimination of alien fragments or chromosomes is possible as observed in *Hordeum* by Kasha and Kao (1970). Conversely, Laurie and Reymondie (1991) and Riera-Lizarazu et al. (1996) have shown that, in similar experiments with wheat and oat pollinated by maize, some partial hybrids were obtained. Maize sequences and chromosomes were conserved as stable additions in oat-maize lines.
(ii) Genome rearrangements consecutive to genomic shock could explain part of the variability observed in hybrid plants (McClintock 1984; Natali et al. 1998).
(iii) Diploidisation and its timing is uncertain. No haploids or aneuploids were observed among our hybrids obtained from mature seed of sunflower pollinated by *H. mollis* (crosses 1 to 11).

An important consequence for the breeder is the necessity to increase the number of crosses made in balance with the number of plants of each hybrid progeny, because each hybrid plant is only partially introgressed and appears to be produced from a unique unpredictable event. The occurrence of interspecific partial hybrids could provide an opportunity to rapidly introgress useful traits of perennial species into a cultivated sunflower background.

The evolutionary consequences of the occurrence of partial hybrids should be considered. Mature seeds of interspecific partial hybrids having undergone a natural process of diploidisation were obtained in this study. If this process can take place in natural conditions, it could lead to limited introgressions between *Helianthus* species.

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