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Evidence for several genomes in *Helianthus*

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Abstract Forty taxa belonging to 36 species and four unclassified accessions of *Helianthus* were studied using RAPD technology. Single ten-mer primers were screened for those amplifying fragments common to several species. We found that when several species shared a common fragment, they belong to the same section of the genus. Moreover, we also found that some fragments are common to all species of the *Helianthus*. Most of the fragments were found to be of the same size in these species and to share the homology indicated by molecular hybridization. Out of 118 retained fragments, 33 were common to all *Helianthus* species, 56 were unique to perennial species of sects. *Atrorubentes* and *Ciliares*, 24 were unique to sect. *Atrorubentes*, 29 were unique to sect. *Helianthus*, whereas 0 were unique to sect. *Ciliares*. Each set of common or specific fragments was assumed to belong to a genome: (1) the **C** genome carrying the fragments common to all species of the three sections, (2) the **H** genome unique to sect. *Helianthus*, (3) the **P** genome common to perennial species (sects. *Atrorubentes* and *Ciliares*), and (4) the **A** genome unique to sect. *Atrorubentes*. The genomic structure was therefore **HC** for sect. *Helianthus*, **CPA** for sect. *Atrorubentes*, and **CP?** for sect. *Ciliares*. Molecular hybridizations with amplification products revealed homologies between *Helianthus* genomes and several other genera in the Helianthinae sub-tribe. The simple method used to characterize these fragments led to powerful tools for recognizing genomes which reconcile the section organization of the genus and the degree of difficulty in crossing perennial and annual forms.

Key words Sunflower · *Helianthus* · Polyploidy · Genomes · RAPD

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Introduction

The *Helianthus* genus belongs to the Asteraceae family, tribe Heliantheae tribe, subtribe Helianthinae, which includes 20 genera with 400 species (Schilling 1997). Anashenko (1979) divided the *Helianthus* per se into seven groups according to genomic relationships (see below). While this classification was certainly well constructed, it unfortunately was not efficient for routine use in classifying any new plants because of the involved cytogenetic studies necessary and the lack of descriptive aspects. Difficulties in crossing sunflower and Jerusalem artichoke with genetic resources could be solved with a better knowledge of genome organization in the genus.

Current *Helianthus* taxonomy is based upon the Gower genetic distances (Gower 1971) calculated on 42 morphologic and phenotypic traits for 49 species. The data were used to compute genetic distances, but the groups have also been arranged taking into account cladistic and biosystematic considerations (Schilling and Heiser 1981). The genus is divided into four sections: *Helianthus* containing 11 species, all diploid $2n = 34$, and *Agrestes* containing the *H. agrestis* diploid species; all these species are annual forms. Sections *Ciliares* and *Atrorubentes* contain perennial species, except for *H. porteri*. *Ciliares* section is divided into two

Table 1 Taxonomy of the *Helianthus* species and related genera of *Helianthinae*

Lane[a] Fig. 1	Lane[b] Fig. 2	Genus	Sections	Series	Species	Subspecies	A/P[b]	*n*[c]	INRA code	PI., USDA code and others[d]
1	1	*Helianthus*	*Helianthus*		*annuus*		A	17	494	PI. 413 154
2					*debilis*	*debilis*	A	17	215	PI. 435 671
3	2				*debilis*	*tardiflorus*	A	17	837	DEB 1564
4	3				*argophyllus*		A	17	92	(FR CLF)
5	5				*neglectus*		A	17	222	PI. 435 768
6	7				*paradoxus*		A	17	206	PI. 435 793
	6				*petiolaris*		A	17	91	(FR CLF)
7					*petiolaris*	*fallax*	A	17	739	PI. 468 822
8	4				*praecox*		A	17	678	PI. 468 851
	39				*niveus*		A	17	–	DNA from USA
9			*Atrorubentes*	*Corona-Solis*	*californicus*		P	51	242	CAL 772
10					*decapetalus*		P	17, 34	551	PI. 468 697
11	14				*divaricatus*		P	17	232	PI. 435 675
12					*eggertii*		P	51	234	PI. 435 677
13					*giganteus*		P	17	553	PI. 468 718
14					*grosseserratus*		P	17	290	(SUNVIR)
15	15				*hirsutus*		P	34	260	HSP 24001
16					*maximiliani*		P	17	568	USDA-1889
17	12				*mollis*		P	17	285	(SUNVIR)
18					*nuttallii*	*nuttallii*	P	17	103	1321 (CLF)
19	13				*resinosus*		P	51	681	RES 1598
20					*salicifolius*		P	17	258	HSP 34004
21	16				*strumosus*		P	34, 51	527	PI. 468 894
22	18				*tuberosus*		P	51	571	USDA-1877
23				*Microcephali*	*glaucophyllus*		P	17	246	GLA 857
24					*laevigatus*		P	34	528	PI. 468 740
25					*microcephalus*		P	17	245	MIC 862
26					*smithii*		P	34	247	SMI 860
27				*Atrorubentes*	*atrorubens*		P	17	252	PI. 435 637
28					*occidentalis*	*plantagineus*	P	17	231	PI. 435 786
29	17				*rigidus*	*rigidus*	P	51	101	(FR CLF)
	35				*porteri*		A	17	–	DNA from USA
30					*silphioides*		P	17	262	HSP 46 001
31	38			*Angustifolii*	*angustifolius*		P	17	529	PI. 468 424
32					*floridanus*		P	17	530	PI. 468 715
33					*simulans*		P	17	564	PI. 468 887
34	9		*Ciliares*	*Ciliares*	*arizonensis*		P	17	203	PI. 435 636
	8–37				*ciliaris*		P	17–51	–	DNA from USA
35	11			*Pumili*	*pumilus*		P	17	227	PI. 435 860
36	10				*gracilentus*		P	17	226	PI. 435 684
37	19		*UC*		*micranthus*		P	17	106	(FR CLF)
38			*UC*		*macrophyllus*		P	?	107	(FR CLF)
39			*UC*		*orgyalis*		P	?	108	(FR CLF)
40	20		*UC*		*sp*		P	?	X	(FR MTP)
	21	*Viguiera*	Sub-genera *Bahiopsis*		*tomentosa*		P	18	–	DNA from USA
	22	*Viguiera*	*Maculatae*		*dentata*		P	17	–	DNA from USA
	23	*Viguiera*	*Maculatae*		*eriophora*		P	17	–	DNA from USA
	31	*Viguiera*	*Diplotischis*		*quitensis*		P	17	–	DNA from USA
	24	*Tithonia*			*rotundifolia*		P	17	–	DNA from USA
	25	*Simsia*			*calva*		P	17	–	DNA from USA
	26	*Pappobolus*			*imbaburensis*		P	17	–	DNA from USA
	28	*Lagascea*			*helianthoides*		P	17	–	DNA from USA
	29	*Alvordia*			*brandegei*		P	17	–	DNA from USA
	30	*Heliomeris*			*multiflora*		P	8	–	DNA from USA
	32	*Iostephane*			*heterophylla*		P	17	–	DNA from USA
	33	*Rhysolepsis*			*palmeri*		P	17	–	DNA from USA
	34	*Almada*			*dentata*		P	17	–	DNA from USA
	36	*Phoebanthus*			*grandiflora*		P	17	–	DNA from USA
	40	*Flourensia*			*cernua*		P	17	–	DNA from USA

[a] Lane numbers in Figs. 1 and 2, respectively
[b] A, Annual; P, perennial
[c] n = Haploid chromosome number
[d] Plant introduction number (PI) and accession origin. FR, France; HSP, California Davis; USA, United States of America; MTP, Montpellier; CLF, Clermont-Ferrand; UC, unclassified species; SUNVIR, Vavilov Institute

series, *Pumili* and *Ciliares*, both containing 6 diploid species, except for *H. ciliaris* 4x or 6x. *Atrorubentes* section contains 31 species which are diploid, tetraploid, or hexaploid, and are divided into four series: *Corona-Solis*, *Microcephali*, *Atrorubentes* and *Angustifolii* (Table 1). The species in one series do not share any common phenotypic traits with those of other series, although the groups are identifiable.

The *Helianthus* genus today includes about 50 species, whereas the *Viguiera* and other related genera (*Heliomeris*, *Scalesia*, *Pappobolus*) have been separated (Schilling et al. 1994). Furthermore, Yates and Heiser (1979) have transferred *H. similis* and *H. ludens* to *Viguiera* (now *V. similis* and *V. ludens*), whereas *H. porteri*, previously *V. porteri*, has been included in section *Atrorubentes*. These rearrangements indicate that the boundaries between these genera are difficult to draw.

Kostov (1939) proposed different genomes for annual and perennial *Helianthus* based on pairing abnormalities observed during meiosis in F_1 plants between sunflower and Jerusalem artichoke. Heiser and Smith (1965) proposed a unique origin of the *Helianthus* common ancestor that it arose by the amphiploid fusion of two genomes with 8 and 9 chromosomes. Anashenko (1979) proposed the first phylogeny for *Helianthus* on the basis of different chromosome sets: the protogenome A for the perennial forms (*Atrorubentes*) of the west coast of North America, the genome B for the annual forms, C for the *Ciliares*, and the protogenome S for perennial shrubs of South America (*Viguiera*). The *Ciliares* group was therefore regarded as a separate group in the *Helianthus*. The genomic organization in *Helianthus* is therefore still questionable.

The random amplified polymorphic DNA (RAPD) polymorphisms in *Helianthus* frequently display fragments of the same size that are common to several species or all species. In order to explain this occurrence we systematically searched for such fragments. We therefore looked for 10-base primers to amplify any fragments (RAPD) in all the species under study and for common fragments present in a taxonomic group which were either section- or series-specific. The distribution of fragments that we observed agrees with the three main taxonomic divisions based on morphologic and phenologic data. There are so many section-specific fragments that we propose a genome structure to explain the results.

Materials and methods

Plant material and plant maintenance

Thirty-six taxa were used covering three sections and six series of *Helianthus*. Four unclassified species were added in an attempt to rank them using molecular markers. The taxon with their taxonomy, the origin, INRA codes, and plant introduction numbers of the accessions are given in Table 1. The plants are currently grown in the experimental field of INRA-Montpellier close to the sea coast in a typical Mediterranean climate. The annual species are maintained by intercrossing ten individuals (hand pollination, flies, or bees) considered as panmictic; the seeds are then collected from each plant (maternal lineage). The perennial forms are maintained in the same way (if enough interfertile plants are present) or through clonal propagules.

DNA isolation

Total DNA for RAPD analyses was isolated from 5 g of frozen mature leaf tissue from one plant according to the method described by Gentzbittel et al. (1994). Purified DNA was quantified either by spectroscopy or on slab gel with lambda DNA as the reference and was adjusted to 10 ng/μl for polymerase chain reaction (PCR) amplification.

RAPD analysis

Random 10-base primers (kits A through E) were obtained from Operon Technologies (Alameda, Calif.) or Bioprobe (Montreuil, France). Amplification reactions (25 μl) were carried out according to Williams et al. (1990) with the following modifications: 40 ng DNA, 40 ng primer, 0.4 U *Taq* DNA polymerase. With a genome size of 10–50 pg/1C (haploid genome DNA content) (Sims and Price 1985; Cavallini et al. 1989; Natali et al. 1993) the amount of genomic DNA used in a RAPD reaction corresponded to between 800 and 4,000 haploid genome equivalents. Amplifications were performed in micro-Eppendorf tubes using a 60-well thermal cycler (Biometra, Eurogentec) with the following temperature conditions: 92°C for 2 min, followed by 35 cycles of 92°C for 1 min; 38°C for 1 min; 72°C for 1 min, and ending with 6 min at 72°C. RAPD products were analyzed by electrophoresis in 2.2% agarose gels stained with 0.2 μg/ml ethidium bromide. The gels were photographed under transmitted UV light using a MP4 Polaroid camera on 9 × 13 cm films. The photographs were enlarged to the actual size of the gels.

Primer screening and notation of fragments

Pre-screening was performed in order to retain those primers amplifying at least one fragment of the same size for a series of plants independent of the taxonomy. Fragments unique to 1 species were not taken into account in this study. Out of the 100 primers assayed, 35 satisfied these criteria. Among these 35 primers, only 21 were systematically used for amplification onto DNAs from the 36 species and the four unclassified accessions when we estimated we had gathered enough data. RAPD fragments were scored for presence (1) and absence (0), and grouped into a matrix based on size homology (GEL). Each RAPD fragment was coded according to the letter of the kit, the number of the primer, and the size of the amplified fragment in base pairs.

RAPD fragment hybridizations

These fragments were isolated from 2.2% agarose gels that were run until an actual separation. The DNA, from either *H. tuberosus* (571) or *H. annuus* (494) as these two species represent standards for those two sections, was recovered through the freeze-squeeze method (Tautz and Renz 1983). Recovered DNA was labelled with α-[^{32}P]-dCTP (111 TBq/mmol) using the Random DNA Primed Labelling Kit from Boehringer. Autoradiograms were obtained with Fuji

Medical X-ray film exposed for a period of a few minutes to a few hours, depending on the signal intensity. The hybridization signals of RAPD fragments, those visible as well as those that were not visible, were named according to the kit, the primer, the species origin for the fragment, either *H. annuus* or *H. tuberosus*, and the size of the fragment hybridizing with the probe. A second matrix based on sequence homology (HYB) was then constructed.

Results

Primer screening and repeatability

In our study, we used 100 single RAPD primers. On average, 6 RAPD fragments per primer were taken into account. Presence was noted 1, regardless of its intensity in a lane, whereas absence was noted as 0. Approximately 600 polymorphic RAPD loci were analyzed, but only on the basis of the following criteria: (1) at least 1 fragment had to be present in more than 1 species, (2) the number of fragments could not exceed 10 per lane, and (3) smears were avoided. Those fragments retained were common to at least 2 species; fragments unique to 1 species were not noted. This method considerably reduced the number of fragments which were used to 88 instead of 600 (13%).

Screening for fragments unique to sect. *Helianthus*

The amplification products obtained with primer C02 are displayed in Fig. 1A. The C02-1,400 fragment (1,400 bp) was present in the *Helianthus*, while there was no band of the same size in any other lanes except for *H. micranthus* (lane 37). In the other gels, 13 fragments amplified from 8 primers characterized the sect. *Helianthus* (Table 2).

Screening for fragments unique to *Ciliares*

Species of this section did not display any specific fragment. The 3 species in the *Ciliares* did not display any of the fragments unique to *Helianthus*, while they displayed 24 fragments common to *Atrorubentes* and *Ciliares* but lacked a specific fragment. In comparison with the two other sections, this lack of unique fragments could not be due to chance.

Screening for fragments unique to sect. *Atrorubentes*

The fragment C02-800 was present in all species belonging to sect. *Atrorubentes*, while there was no band

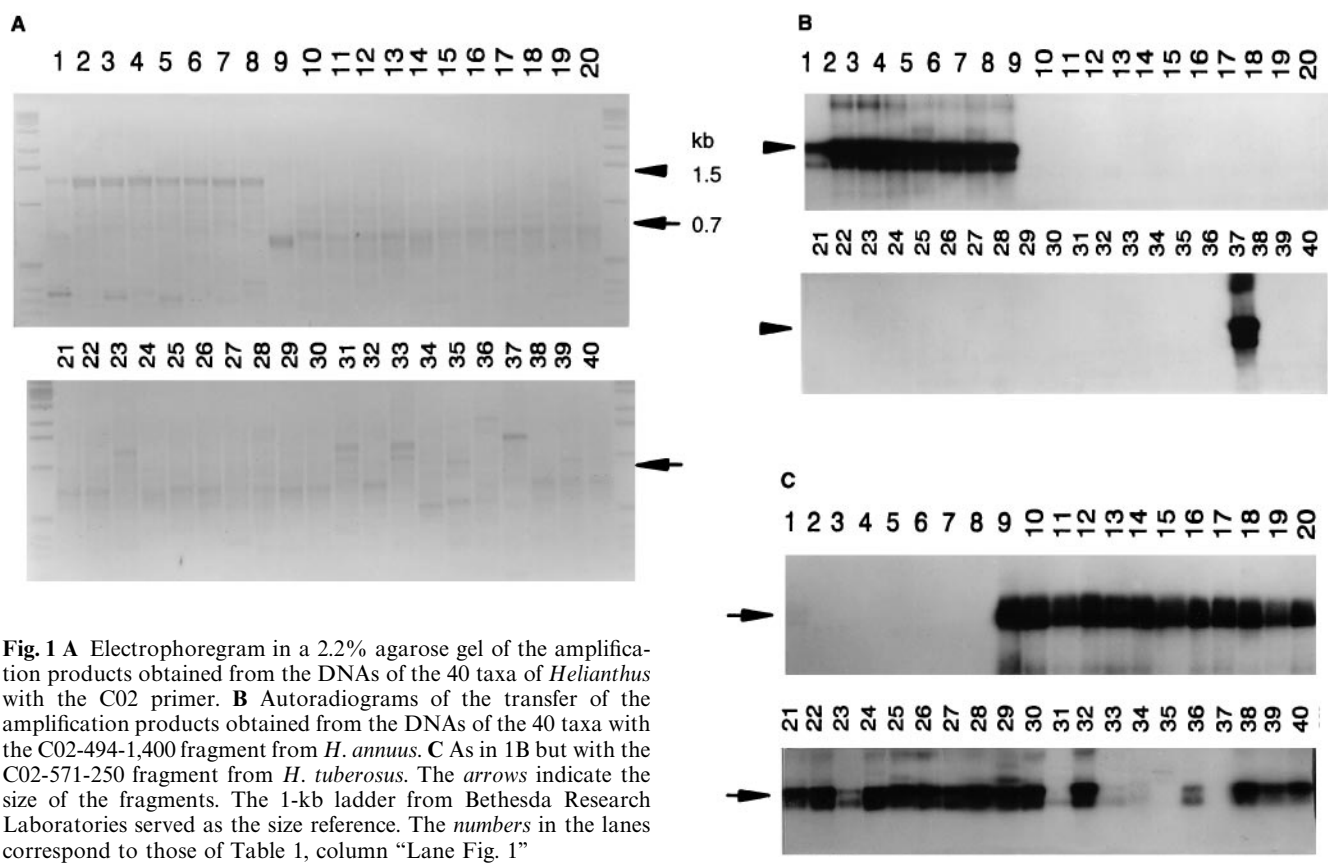
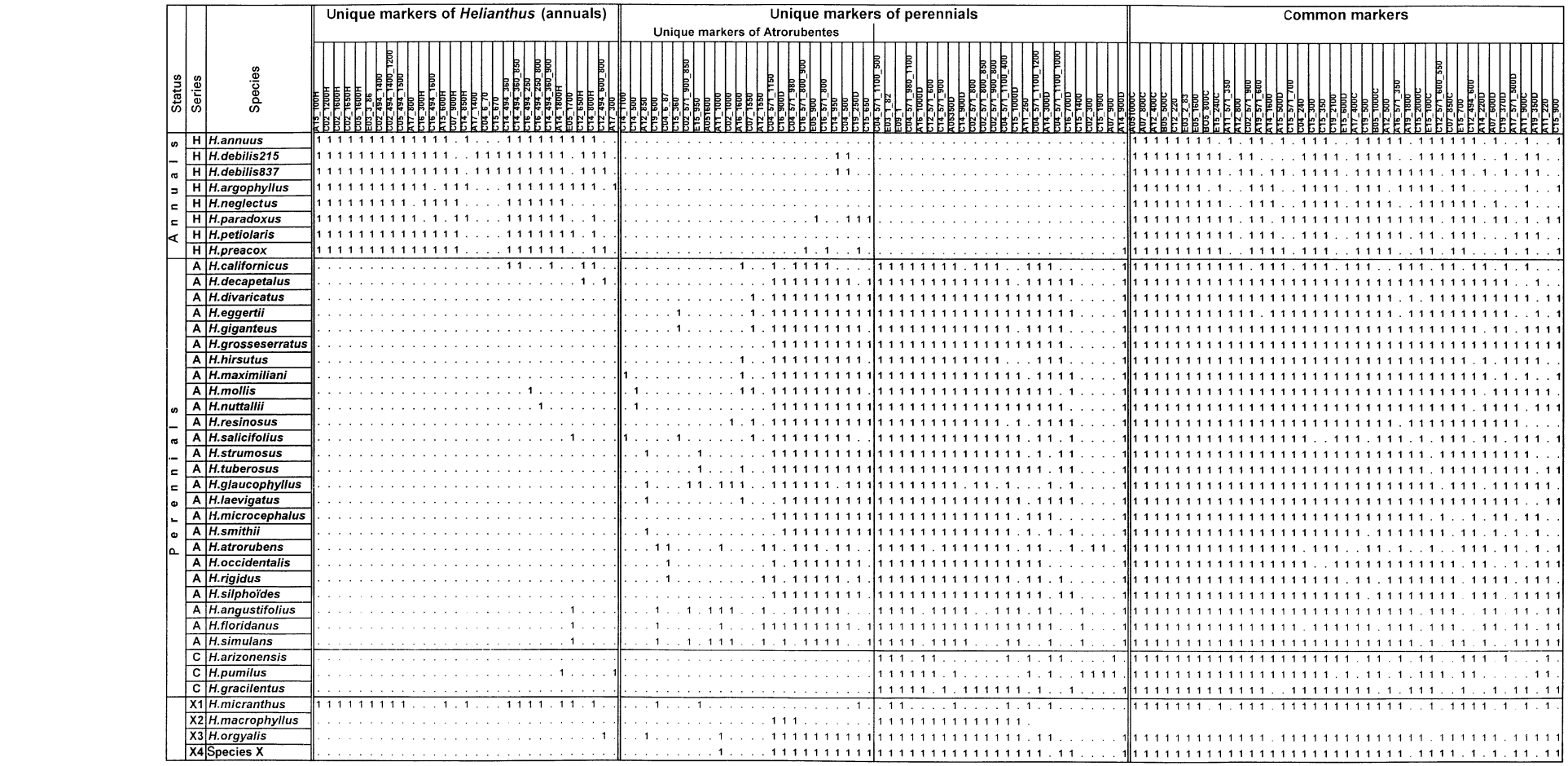


Fig. 1 A Electrophoregram in a 2.2% agarose gel of the amplification products obtained from the DNAs of the 40 taxa of *Helianthus* with the C02 primer. **B** Autoradiograms of the transfer of the amplification products obtained from the DNAs of the 40 taxa with the C02-494-1,400 fragment from *H. annuus*. **C** As in 1B but with the C02-571-250 fragment from *H. tuberosus*. The *arrows* indicate the size of the fragments. The 1-kb ladder from Bethesda Research Laboratories served as the size reference. The *numbers* in the lanes correspond to those of Table 1, column "Lane Fig. 1"

Table 2 SCO Matrix of presence (1) versus absence (·) after correction of data for fragment amplified with 21 primers. The designation of the fragment is followed by the size of the primer (in bp); the designation of the hybridization fragment is followed first by the origin of the primer probe (494 = *H. annuus* and 571 = *H. tuberosus*) and then by its size (in bp)



at the same position in any of other lanes. In the other gels, 20 fragments amplified from 14 primers characterized sect. *Atrorubentes* (Fig. 1A, Table 2). Three of the unclassified species, *H. macrophyllus*, *H. orgyalis*, and *H. sp.*, displayed the same pattern as the *Atrorubentes* (Fig. 1C, lanes 38–40).

Screening for fragments common to sects. *Atrorubentes* and *Ciliares*

Fifty-five fragments were common to species belonging either to sect. *Ciliares* or to sect. *Atrorubentes.*

Screening for fragments common to all *Helianthus* species

We detected fragments which were always present no matter what species was evaluated (Table 2).

Characterization of the fragments

The fragments amplified from 1 primer from the DNAs of the 36 species were separated on one gel. On the basis of one migration we held that fragments that migrated to the same position were homologous, although it is likely that an homology in size could not correspond to an homology in sequence. To check the homologies we systematically isolated the fragment in either *H. annuus* (494) for the sect. *Helianthus* or *H. tuberosus* (571) for the sect. *Atrorubentes.* The hybridization signals obtained from the transfer of the RAPD gel corresponded here to non-visible RAPD fragments, but not to restriction fragment length polymorphisms (RFLPs). The hybridization signals were clearly detected in all the *Helianthus* with the C16-494-1,600 fragment as a probe, while they were not detected in any of the other lanes (not shown). They were detected in all the *Atrorubentes* with the C14-571-900 probe, but they were not detected in any of the other lanes (not shown). We did not find any specific fragment which was not hybridized by the reference fragment as a probe in one section, and, except for *H. gracilentus*, the 350-bp fragment did not hybridize the A16-571-350 probe (not shown).

Primer OPA19 allowed the amplification of fragment OPA19-600 which was common to all 40 species. All amplification products were hybridized with the reference fragment OPA19-571-600, which demonstrated that a homologous fragment was present in each species except *H. debilis* (not shown). A fragment common to all the *Atrorubentes*, A11-571-350, was also present in the *Ciliares* (not shown).

Data presentation

All in all, 88 positions of fragments were noted. The GEL matrix contained 36 lines and 88 columns (GEL36). A second matrix plus the 4 unclassified species was also constructed and noted as GEL40. Each of 25 reference fragments was used separately as a probe onto the corresponding Southern transfers of the gels. The reference fragments revealed 37 signals because several fragments were hybridizing with 1 reference fragment as a probe. The HYB matrix contained 36 lines and 37 columns (HYB36). Both two matrices of data used (1) to indicate the presence of a fragment or a signal and (0) for absence of a fragment or signal (not shown). The difference between the GEL and the HYB scores was due to the fact that the HYB score was not a sub-sample of the GEL score, because 1 probe hybridized not only with visible fragments but also with undetected fragments. The C02-494-1,400 fragment as a probe hybridized (Fig. 1B, lanes 1–8) with the 1,400-bp visible (Fig. 1A, lanes 1–8) fragment and with the 1,200-bp non-visible fragment (Fig. 1A) We therefore constructed a new score matrix (SCO) containing the GEL scores and the specific information of the HYB scores. The SCO matrix contained either 36 or 40 lines and 118 columns. In this matrix we ordered the fragments according to their presence in annuals, in perennials, and common to all species.

Genome identification

The fragments studied (118) were either common to all species of the three sections (33) or specific to sect. *Helianthus* (29). In *Atrorubentes* and *Ciliares* we found fragments common to perennial species (32), whereas 24 were specific for Atrorubentes (Table 2).

Fragments found in *Helianthinae*

We used the primers that allowed the amplification of fragments specific to one section to amplify DNA from other species of sect. *Helianthus* (*H. niveus*), sect. *Agrestes* (*H. agrestis*), sect. *Atrorubentes* (series *Atrorubentes*: *H. angustifolius*; series *Divaricati*: *H. porteri*), sect. *Ciliares* (*H. ciliaris*), and other genera (*Viguiera*, *Tithonia*, *Simsia*, *Pappobolus*, *Lagascea*, *Alvordia*, *Heliomeris*, *Iostephane*, *Rhysolepis*, *Aldama*, *Phoebanthus*, and *Flourensia*). As controls we used other previously studied *Helianthus* species belonging to sect. *Helianthus*, sect. *Atrorubentes*, and sect. *Ciliares* (Table 2).

The profiles for *H. angustifolius* corresponded to those of other *Atrorubentes* species. However, for other genera the profiles were too different to allow fragment recognition. Therefore, we only used fragments amplified specifically in sect. *Helianthus*, sect. *Atrorubentes*,

or common to all species as a probe to hybridize the corresponding Southern transfers. This revealed:

1) fragments from the H genome. The RAPD profiles for *H. niveus* corresponded to those of other sect. *Helianthus* species. C16-494-250 hybridized 2 fragments (250 bp and 350 bp) with sect. *Helianthus* (annual species) and also with *V. Dentata (strong signal)* and *Alvordia (faint signal)*, but not with the other *Viguiera*. It also hybridized with another fragment of about 700 bp in *H. agrestis* (annual form sect. *Agrestes*), in *H. porteri* (annual form, sect. *Atrorubentes*), and with *Phoebanthus grandiflora* (Fig. 2). C04-494-220 (not shown) hybridized the 220-bp fragment in *H. niveus*, *H. agrestis*, *Viguiera tomentosa*, *Tithonia rotundifolia*, *Iostephane heterophylla*, but it was lacking in *H. porteri* and *H. micranthus*.
2) fragments from the A or P genome. Other results not illustrated include C04-571-1,000 hybridizing 2 fragments (1100 bp and 1000 bp) in all sect. *Atrorubentes* species except *H. porteri*. In *H. porteri*, it hybridized 1 fragment, 1050 bp) and the same signal was found in *H. ciliaris* and in *Viguiera eriophara*, whereas there was no signal for *H. agrestis* and the two other *Viguiera*. Two fragments of 1100 bp and 1000 bp were also hybridized in *Pappobolus inbaburensis* and in *Iostephane heterophylla*. Two faint signals (1000 and 900 bp) were present in *H. argophyllus*, *H. praecox* and *H. neglectus*. C02-571-700 strongly hybridized 3 fragments (700 bp, 800 bp, 950 bp) with all the *Atrorubentes*. There were faint signals with all the *Ciliares* at 650 bp and 750 bp. There was also a signal in *H. annuus* but not in *H. micranthus*. Very faint signals were detectable for *Alvordia brandegei* and *Tithonia rotundifolia*. A11-571-1,000 hybridized a fragment (1,000 bp) in all the *Atrorubentes*, it also hybridized an equivalent fragment in the *Ciliares*. C04-494-240 was present *H. porteri*, *H. ciliaris*, *V. tomentosa*, *Tithonia* and *Iostephane*.
3) fragments from the C genome. C15-571-700 hybridized 2 fragments (700 bp, 600 bp) in sects. *Helianthus*, *Atrorubentes*, and *Ciliares*, but not for *H. agrestis* (400 bp) and *H. porteri* (400 bp). C12-571-600 hybridized 2 fragments (600 bp and 550 bp)) in all of sect. *Atrorubentes* and in sect. *Ciliares* except *H. pumilus*. The 550-bp fragment was also present in most sect. *Helianthus* species. Faint fragments were detectable in *Viguiera dentata* (650 bp, 700 bp), *Tithonia* (700 bp, 650 bp), *Simsia* (650), and *Pappobolus* (700 bp). C02-571-700 strongly hybridized 3 fragments (700 bp, 800 bp, 950 bp) with all the *Atrorubentes*. There were faint signals with all the *Ciliares* at 650 bp and 750 bp. There was also a signal in *H. annuus* but not in *H. micranthus*.

Discussion

Our approach was to establish molecular relationships in *Helianthus* in order to easily assign an individual to a taxon. We revealed that some species are hybrid between annual and perennial forms (such as *H. micranthus*) and this enabled us to propose three genomes. Most of markers were specific to sections. Only 40 species were available in Montpellier due to the fact

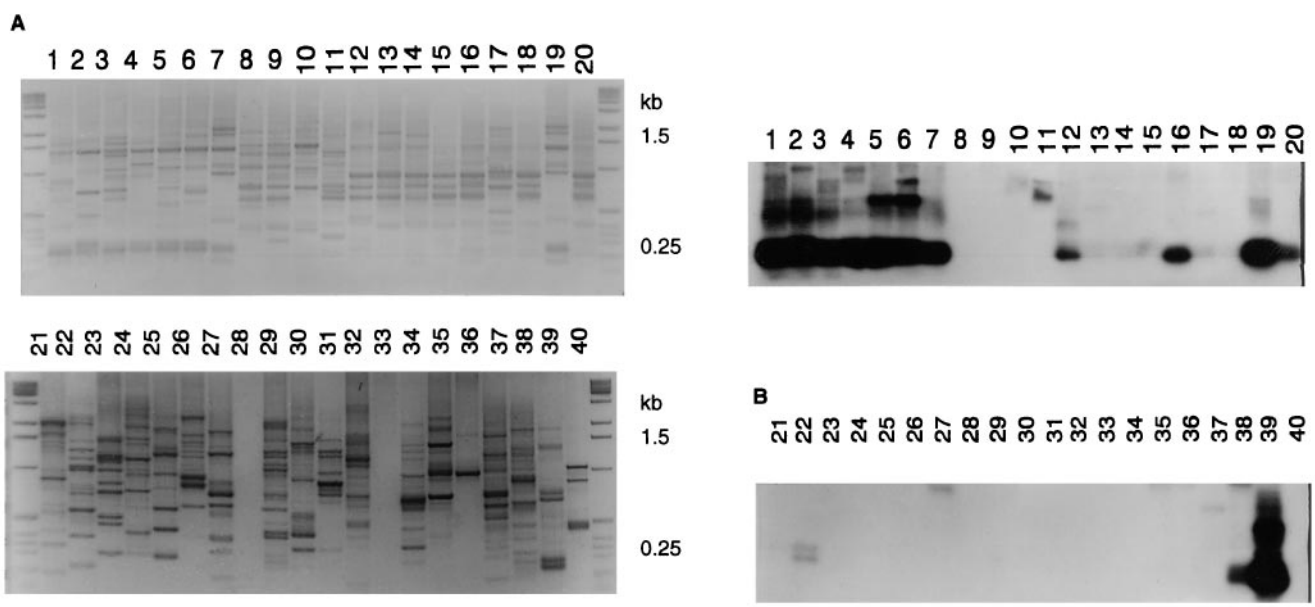


Fig. 2 A Electrophoregram in a 2.2% agarose gel of the amplification products obtained from the DNAs of *Helianthus* and related genera with the C16 primer. **B** Autoradiogram of the transfer of the amplification products obtained from the DNAs of the 40 taxa with the C16-571-250 fragment from *H. tuberosus*. The *numbers* in the lanes correspond to those in Table 1, column "Lane Fig. 2". The 1-kb ladder from Bethesda Research Laboratories served as the size reference

that *Helianthus* is native to North America and the remaining species are difficult to maintain in Montpellier. However, we verified that *H. niveus* (sect. *Helianthus*) and *H. angustifolius* (sect. *Atrorubentes*) carry the expected RAPD fragments. Moreover, we determined that *H. agrestis* (sect. *Agrestes*) is different from other annual species but closer to *H. porteri* than expected from the taxonomy according to a few RAPD fragments. The distribution of RAPD fragments suggested the presence of three different genomes. These genomes were also found in related genera belonging to the Helianthinae subtribe.

Our method for screening primers appeared to be very efficient for characterizing RAPD fragments conserved among species which have been separated for about 10 million years (Graham 1996). Since the screening reduced the sample of retained fragments, we wondered whether the interpretations of the results might have been biased. Twenty-one primers led to several hundred fragment positions. The characterization of the fragments was not realistic. Most of the fragments were expected to be polymorphic between plants of 1 species, while those conserved for a section should be present in all plants of that section and without any polymorphism of 1 species belonging to this section; this was verified across experiments. Consequently, the choice of only one individual per species was justified as the individual chosen had no bearing on the scoring of fragments. Furthermore, we observed that closely related species frequently displayed fragments of the same size in comparison with those of distant species. This cannot be due to chance alone, and therefore we would stress the logic of our design when interpreting the data. The second point was the unexpected synergy between the GEL and the HYB scores. The HYB score was expected to be a sub-sample of the GEL score supposing that RAPD fragments were independent from one another, with just some adjustments between species. Since 1 probe hybridized not only the visible fragments but also undetected fragments, we studied a new score matrix (SCO) combining the GEL and the specific information from the HYB scores. The two matrices contained either 36 or 40 lines and 118 columns. Visible and non-visible fragments could be due to the fact that amplification occurred in an array of direct and forward tandemly repeated sequences carrying the primer sequence, leading to several fragments of various lengths but sharing a common sequence, which in turn resulted in several hybridization signals.

We propose that the four sets of fragments unique to *Helianthus*, unique to *Atrorubentes*, common to *Atrorubentes* and *Ciliares*, and common to all species correspond to four genomes noted **H**, **A**, **P**, and **C**. The *Helianthus* should be **CH**, the *Atrorubentes*, **CPA**, and the *Ciliares*, **CP?** with compensation for $n = 17$. We suggest that the **P** genome or part of the **P** genome was doubled. These four genomes imply that recognizing the three sections with RAPD fragments may reveal more subtle variations than morphology. Those fragments present in all species of one section and found in one or a few species of another sections would be due to ancient crosses (introgressions). Formally, the **P** and **C** genomes could have been rearranged as a deletion and duplication for sect. *Ciliares* (Table 3). *H. agrestis* may carry a variant **H**, genome **H′**, since it displays a quite different genome size than in sunflower (7.22 pg), Sims and Price (1985) have measured 20.6 pg. We suggest that it is also partly present in *H. porteri.*

Table 3 Genomic organization in sects. *Helianthus*, *Ciliares* and *Atrorubentes* and related genera of Helianthineae

Genomes	*C*	*H*
C		*Helianthus*
P ?	*Ciliares*	
P A	*Atrorubentes perennial*	
P A′	*Atrorubentes H. porteri*	
H′	*Agrestes H. agrestis*	
?[a]	*V. Eriophora*	*V. dentata*
	Pappobolus	*Alvordia*
	Iostephane	
	Tithonia	
	V. tomentosa	

[a] Not yet published

Our results using more *Helianthus* species are consistent with those obtained previously for the three main *Helianthus*, *Atrorubentes* and *Ciliares* sections. *Helianthus niveus*, *H. angustifolius*, *H. ciliaris* were ranked as expected. *Helianthus agrestis* was different from both species of sect. *Helianthus* and sect. *Atrorubentes*: this justifies its position in a specific section. However, we noticed that *H. porteri* was not homogeneous with other *Atrorubentes.* It displayed fragments similar to those of *H. agrestis*, thus the RAPD enabled us to classify it as being close to *H. agrestis.* This is in agreement with its annual status among perennial species. We attributed for both species provisional A′ and H′ genomes, respectively.

The same primers applied to related genera have revealed a close relationship between different genera of the subtribe Helianthinae. This has already been suggested for *Helianthus* and *Viguiera* (Schilling and Jansen 1989), *Iostephane* and *Viguiera* (Schilling and Panero 1991), and *Pappobolus* (Ex *Helianthopsis*) and *Helianthus.* While we have not adequately sampled all these genera and our results cannot establish strict relationships, the RAPD fragments appeared powerful enough to reveal possible phylogeny (Sossey-Alaoui et al. in preparation). However, it remains to be explained why several RAPD fragments lacking in *Ciliares* are found in *Atrorubentes.* The absence of these fragments was confirmed in *H. ciliaris.* This will have to be checked further and we present here a preliminary diagram (Table 3); we suggest possible translocations

followed by the loss of some chromosomes and doubling others. The amphiploid origin of the basic genome allowed such events to lead to viable genomic structures.

Polyploidy has been widely reported in several genera: *Arachis* (Singh 1988), *Brassica* (Song et al. 1995), *Triticum* (Zohary and Feldman 1962), and synthetic polyploids obtained by fusing genomes of diploid ancestor species supported such an origin in *Brassica* (Singh 1988; Song et al. 1995). This type of situation has not yet been reported in the Compositae although polyploidy has been reported in several genera: *Taraxacum*, *Helianthus* (Lane et al. 1996). In this family the chromosome numbers are widely different, $n = 2$ and 3 for *Machaeranthera* and *Crepis*, respectively (Lane et al. 1996), and $n = 51$ for some *Helianthus* (Schilling and Panero 1996), but also widely different for species belonging to one genus (*Heliomeris*, *Viguiera*, *Helianthus*). This can be explained by polyploid series. Usually chromosomal rearrangements, amphiploidy, unreduced gametes during meiosis are the mechanisms proposed to explain such a wide variation in the number of chromosomes (Den Nijs and Menken 1996). The wide variation in fertilization mechanisms reported in this family (apomixis, apogamy, agamy) should create and maintain any new genomes resulting from polyploid events. The Helianthinae are only found in America, in soils stressed by drought, cold and salinity. This could explain the survival of polyploid species more adapted to survival in stressed soils (Song et al. 1995). All the genera of the Helianthineae subtribe carry 18 or 17 chromosomes except for some Heliomeris species with $n = 8$. This would mean that different polyploid events occurred between species with 8 and 9 chromosomes, but no species with $n = 9$ is known in the subtribe of Helianthineae.

The main fact emerging from this study deals with the RAPD fragments of the same size and sharing a strong homology which appeared as key fragments in genome definition. Recent studies carried out with the RAPD cloned fragments have shown that they correspond to repeated sequences. It is therefore important to determine why RAPDs are so efficient compared to other molecular markers in recognizing different genomes.

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