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MOLECULAR RELATIONSHIPS OF *Helianthus* BASED ON RAPD MARKERS

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SUMMARY

Phenetic and phylogenetic relationships in genus *Helianthus* were assessed by molecular markers. Forty taxa belonging to 36 species and 4 unclassified accessions of *Helianthus* were studied using the RAPD technology. Single ten mer primers were screened for those amplifying fragments common to species belonging to one section or common to all species of the genus. Sequence homology between RAPD fragments of the same size was confirmed by molecular hybridization. Most of the fragments were found to be of the same size in other 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 which 24 were unique to sect. *Atrorubentes*, 29 were unique to sect. *Helianthus*, whereas 0 were unique to sect. *Ciliares*. The presence/absence of RAPD (fragments or signal hybridizations) were used to determine all structures using correspondence analysis and to compute genetic distances. Correspondence analysis clearly separated the 3 sections and recognized some series. The Jaccard distance and the Sokal and Michener similarity were chosen with UPGMA and Neighbor Joining Method to construct phenetic trees which were very similar to those of the current taxonomy. The simple method used to characterize these fragments led to powerful tools to use RAPD for help in taxonomy.

Key words: ***Helianthus*, taxonomy, genomes, RAPD, evolution**

INTRODUCTION

The *Helianthus* genus belongs to the *Asteraceae* family, to the *Heliantheae* tribe, to the *Helianthinae* subtribe including 20 genera with 400 species (Schilling, 1994). A group of species belonging today to different genera was first brought together in the taxonomy proposed by De Candolle (1836). He has proposed five

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classes: 1) the annual forms (*Annui*); three classes (2, 3 and 4) of perennial based upon the leaf patterns (alternate, opposite) and the head color, yellow, orange and red, respectively; 5) the *Fruticosi* corresponded to shrubs from Mexico and South America, which are now named either *Viguiera* or *Helianthopsis*. This taxonomy has been frequently revised. Torrey and Gray (1842) have excluded the *Fruticosi* and distinguished 6 sections for the *Helianthus*: *Annui*, *Angustifolii*, *Atrorubentes*, *Laetiflora*, *Corona-Solis*, and *Microcephali*. Gray (1884) proposed to group the annual forms in one section and all the perennial forms in another. Watson (1929) also divided the genus into two sections, but his taxonomy was based upon the head color which is no longer considered as a distinctive characteristic. Heiser *et al.* (1969) took into account the presence and the morphology of underground organs (rhizome or tuber) and the ability of intercrossing species to divide the genus into three sections: *Annui*, *Atrorubentes* and *Ciliares*. Anashenko (1979) proposed the first phylogeny for *Helianthus* according to 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*.

Current *Helianthus* taxonomy is based upon the Gower genetic distances (Gower, 1971) calculated on 42 morphologic and phenotypic traits for forty-nine 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 $2n=34$, *Agrestes* containing the *H. agrestis* diploid species. All these species are annual forms, whereas sections *Ciliares* and *Atrorubentes* contain perennial species, except *H. porteri*. *Ciliares* section is divided into two series: *Pumilii* and *Ciliares*, both containing 6 diploid species except *H. ciliaris* 4x or 6x. *Atrorubentes* section contains 31 species which are diploid, tetraploid or hexaploid, and are divided into 4 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. This is a consequence of the phenetic method used, therefore these groups may not have a monophyletic origin. Methods based upon parsimony or cladistic approaches must be used to construct phylogenetic trees. In this taxonomy, *Ciliares* appeared as a sub-group of the *Atrorubentes* in contrast to the Anashenko (1979) model.

It appears that several criteria have been used to recognize taxa in the genus, including traits such as herb, bush, shrub plant, rhizome, hairiness, morphology, disposition, or color and roots, stems, leaves, heads, rays, disks, phyllaries, petioles, and achenes. More recently, trichomes unique to section *Atrorubentes* have been reported (Schilling *et al.*, 1994). In the past, several genera have been grouped in *Helianthus*. The bush and shrub perennial species today belong to the genus *Viguiera* including about 200 species native to South America.

Table 1: Taxonomy of the forty specimens. A/P=Annual or Perennial, *n*=haploid chromosome number, INRA code, PI=plant introduction number and accession origin: YUG=Yugoslavia, FR=France, HSP=Spain, USA=United States of America, MTP=Montpellier, CLF=Clermont-Ferrand, CO=Cordoba, UC= unclassified species

Lane	Section	Series	Species	Subspecies	A/P	*n*	INRA code	PI,USDA code and others
1	*Helianthus*		*annuus*		A	17	494	PI. 413 154
2			*debilis*	*debilis*	A	17	215	PI. 435 671
3			*debilis*	*tardiflorus*	A	17	837	DEB TAR 1564
4			*argophyllus*		A	17	92	(FR CLF)
5			*neglectus*		A	17	222	PI. 435 768
6			*paradoxus*		A		206	PI. 435 793
7			*petiolaris*	*fallax*	A	17	739	PI. 468 822
8			*praecox*		A	17	678	PI. 468 851
9	*Atrorubentes*	*Corona-Solis*	*californicus*		P	51	242	CAL 772
10			*decapetalus*		P	17,34	551	PI. 468 697
11			*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			*hirsutus*		P	34	260	HSP 24001
16			*maximiliani*		P	17	568	USDA-1889
17			*mollis*		P	17	285	(SUNVIR)
18			*nuttallii*	*nuttallii*	P	17	103	1321 (CLF)
19			*resinosus*		P	51	681	RES 1598
20			*salicifolius*		P	17	258	HSP 34004
21			*strumosus*		P	34,51	527	PI. 468 894
22			*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			*rigidus*	*rigidus*	P	51	101	(FR CLF)
30			*silphioides*		P	17	262	HSP 46 001
31		*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	*Ciliares*	*Ciliares*	*arizonensis*		P	17	203	PI. 435 636
35		*Pumilii*	*pumilus*		P	17	227	PI. 435 860
36			*gracilentus*		P	17	226	PI. 435 684
37	UC		*micranthus*		P	?	106	(FR CLF)
38	UC		*macrophyllus*		P	?	107	(FR CLF)
39	UC		*orgyalis*		P	?	108	(FR CLF)
40	UC		*sp*		P	?	X	(FR MTP)

However, the advantages of such a taxonomy are numerous. The taxonomy is based upon genetic distances which are established using numerous traits. This fact may explain the four sections, two perennial and two annual. The taxonomy is a convenient tool for most botanists. There is no discrepancy between the taxonomy and the biological, ecological and evolutionary knowledge available for this genus. The difficulties of making crosses between the two groups of annual and perennial species are a problem for breeders. The taxonomy is therefore a convenient tool to predict the success of most interspecific crosses (Serieys, 1984).

Taxonomy based on DNA polymorphisms is directly applicable to all plants and does not suffer from some of the problems inherent of phenetic taxonomy, due to the use of (1) genetic distances which are abstract, (2) the complexity of the phenotypic traits which may not be observed in one plant, (3) one extra plant cannot be assigned in the taxonomy by scanning the traits since the hierarchic classification (data / genetic distances / aggregating method) involves reconstructing one phenetic tree, (4) the morphological traits are environment sensitive and moreover may exist in different states, thus it is difficult to determine the ancestor state, and (5) there are also several unclassified species or accessions which have not been used by Schilling and Heiser (1981).

Since none of the marker types and methods, which have been used to establish relationships between *Helianthus* species, has led to a classification in agreement with the taxonomy, we wonder whether this could be overcome by using another type of molecular markers recognized as targeting mainly the repetitive DNA and, secondarily non-repetitive DNA. We therefore screened 10-b 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.

We used RAPD's to structure the polymorphisms in the genus. We computed the correspondence analyses, the genetic distances between species and the phylogenetic analyses based upon parsimony. The results agree with the taxonomy established by Schilling and Heiser (1981) based on morphological and phenological data with few exceptions which are discussed below. Moreover, a genome structure is proposed to explain the results.

MATERIALS AND METHODS

Data analysis, genetic distances, phenograms and phylograms

Correspondence analysis is adapted to look for contingency tables, in this case the matrix of presence / absence of fragments or signals (Benzécri, 1973; Goodman, 1988; Greenacre, 1984). The analyses were carried out using the CORRESP procedure of SAS package Inc. Technologies (1992) according to the process described by Peltier *et al.* (1995). Correspondence analysis applied to the matrix of GEL data was used to look for any structure of the species while correspondence analysis applied to the fragments was used to determine those mostly responsible for the

preceding structure. The matrix of genetic distances was computed with Jaccard distance (JD) or the Sokal and Michener dissimilarity (SMD) either on GEL or HYB according to Jaccard (1908) and Sokal and Michener (1958), respectively. The distances were aggregated with the unweighted pair grouping with mean average method (UPGMA) (Sneath and Sokal, 1973) and the Neighbor Joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988). Construction of phenograms was made with the Drawtree from Phylip package. We used the Neighbor and Drawgram programs from Phylip (Felsenstein, 1989) to compute and to draw the phenograms. We used the Wagner parsimony analysis (Eck and Dayhoff, 1966; Kluge and Farris, 1969) on the SCO matrix to propose phylograms for wild species. We did not use either the bootstrap resampling method (Felsenstein, 1985) or the Jackknife method because these two methods implicate that the traits evolved independently, hypothesis not demonstrated for RAPD profiles. We used the Mix and Drawtree programs from Phylip Software (Felsenstein, 1989) to compute and to draw the phylogenetic trees.

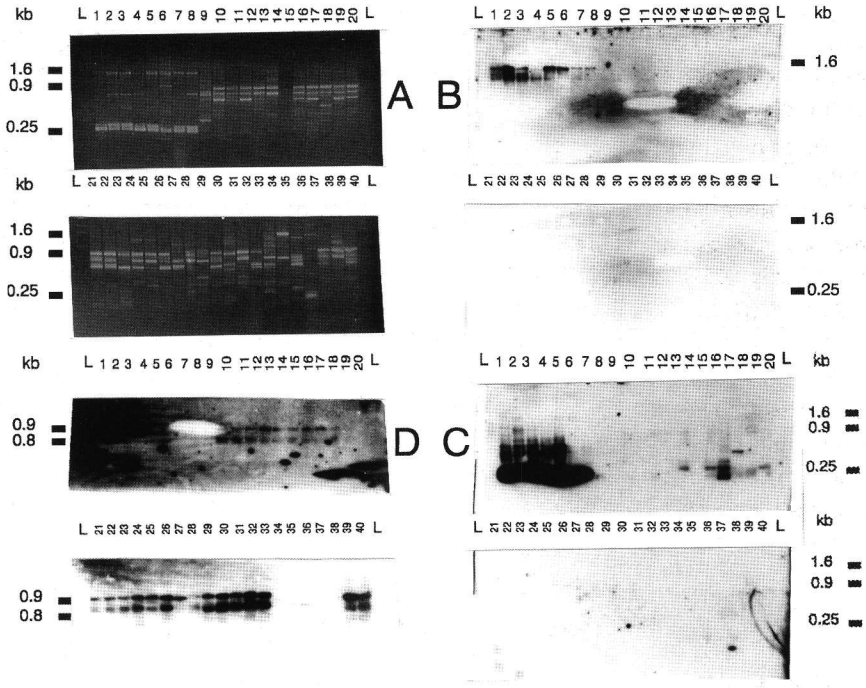


Figure 1: ***A:*** *Electrophoregram in a 2.2% agarose gel of the amplification products obtained from the DNAs of the 40 taxa of Helianthus with the C16 primer.* ***B:*** *Autoradiograms of the transfer of the amplification products obtained from the DNAs of the 40 taxa with the C16-494-1-600 fragment from H. annuus.* ***C:*** *with the C16-594-250 fragment from H. annuus.* ***D:*** *with the C16-571-900 fragment from H. annuus. The arrows indicate the size of fragments. The 1 kb ladder from Bethesda Research Laboratories served as reference for size of fragments. The numbers in the lanes correspond to those of Table 1.*

RESULTS

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 twenty-five 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 one reference fragment as a probe. The HYB matrix contains 36 lines and 37 columns (HYB36). The two matrices of data contain (1) for presence of a fragment or a signal or (0) for absence of a fragment or a 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 one probe hybridized not only with visible fragments but also with undetected fragments. The C02-494-1,400 fragment as a probe hybridized (Figure 1B, lanes 1-8) with the 1,400 bp visible (Figure 1A, lanes 1-8) fragment and with the 1,200 bp non-visible fragment (Figure 1A). We therefore constructed a new score matrix (SCO) containing the GEL scores and the specific information of the HYB scores. The matrix contains either 36 or 40 lines and 118 columns. All further analyses were performed on these matrices only.

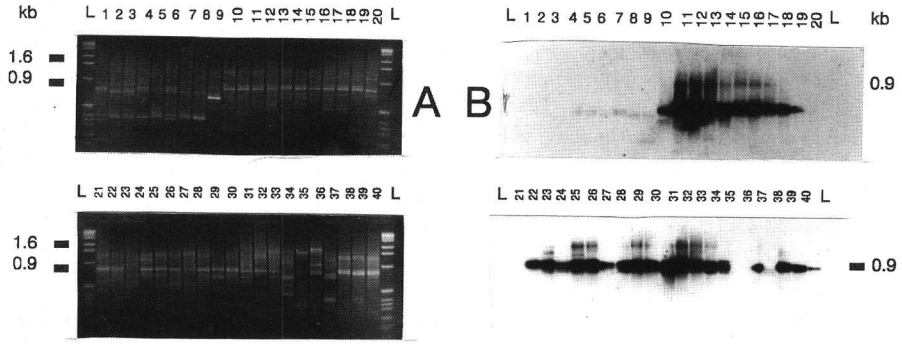
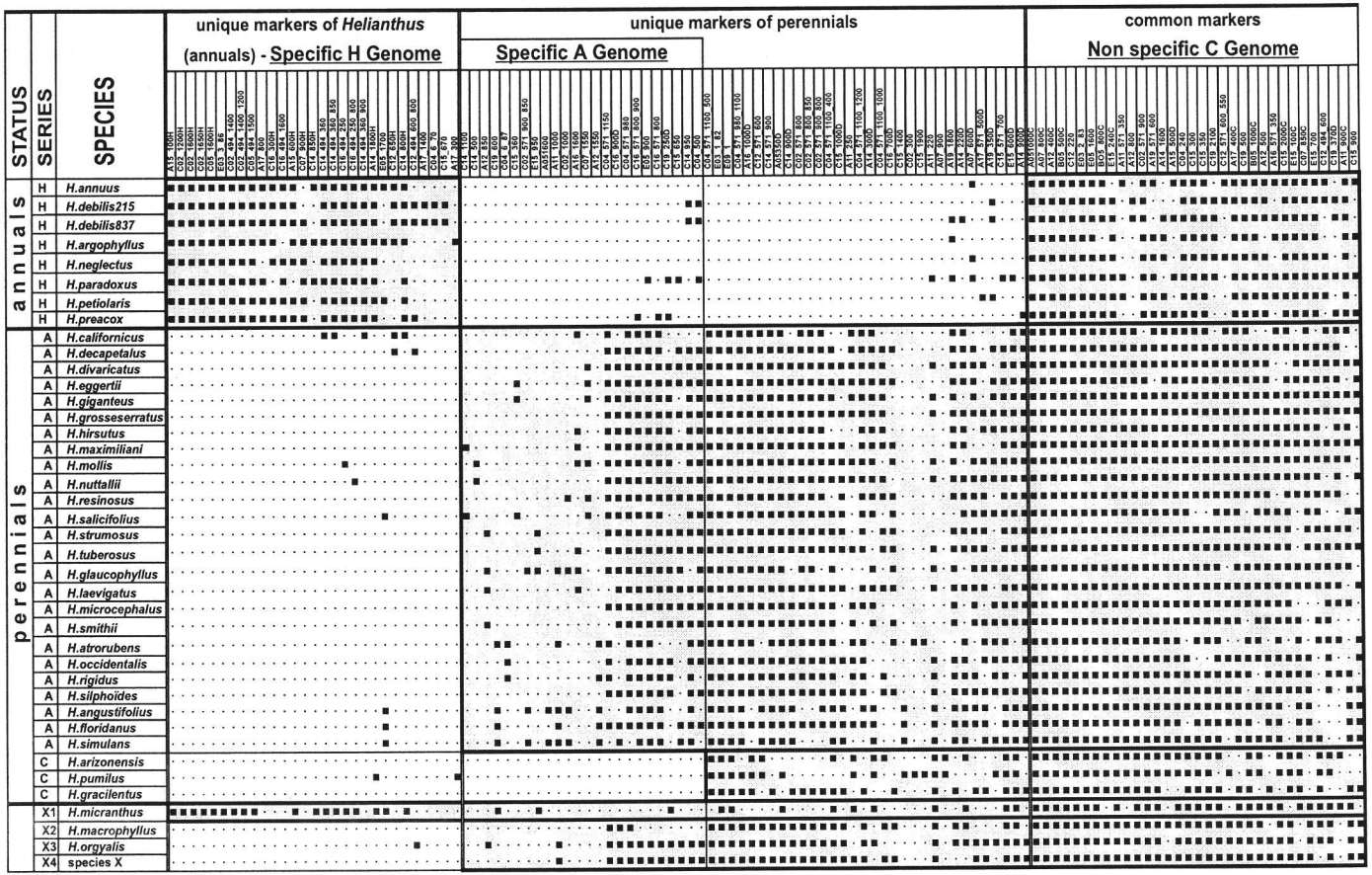


Figure 2: ***A:*** *Electrophoregram in a 2.2% agarose gel of the amplification products obtained from the DNAs of Helianthus and related genera with the C14 primer.* ***B:*** *Autoradiogram of the transfer of the amplification products obtained from the DNAs of the 40 taxa with the C14-571-900 fragment from H. tuberosus. The numbers in the lanes correspond to those in Table 1. The 1 kb ladder from Bethesda Research Laboratories served as reference for the size of fragments.*

Data analysis

The correspondence analysis performed on SCO (Figure 2) with species as objects clearly separates the annual from the perennial forms (40% of importance for Dim 1), and both series of sect. *Ciliares* from sect. *Atrorubentes* (Dim 3), series *Angustifolii* is separated (9% of importance for Dim 2), whereas the unclassified species *H. micranthus* is intermediary between the annual forms and the perennial forms. The three other unclassified species were grouped with sect. *Atrorubentes*. In consequence, correspondence analysis enables us to identify the 3 sections and

Table 2: Sorted SCO Matrix of presence (■) absence (.) after correction of data for fragment amplified with the 21 primers. Designation of fragment: primer-size in bp; after hybridization: primer-probe origin, 494 = *H. annuus* or 571 = *H. tuberosus* - size in bp.



the *Angustifolii* among the *Atrorubentes*. Correspondence analysis with markers as objects indicates which markers are specific for the preceding groups and that series *Ciliares* and *H. micranthus* did not display specific markers (not shown, Table 2).

Phenogram comparisons

The distances are always greater between the annual and perennial forms and among each group, on average, the distances between the annual are shorter than between the perennial forms. The *Ciliares*, as well as *H. micranthus*, appear as intermediary between the annual and the perennial species, either with JD (not shown) or SMD and the NJ method (Figure 3).

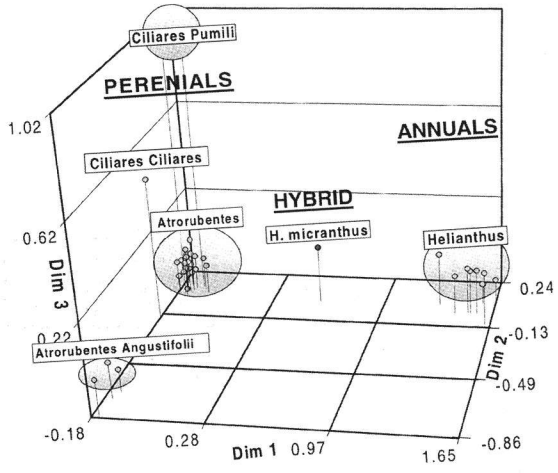


Figure 3: Correspondence analysis performed on the corrected matrix with corrected data with species as variables.

DISCUSSION

Our approach was to establish a molecular taxonomy of *Helianthus* in order to easily assign an individual to a taxon which is more important than establishing a molecular phylogeny. This taxonomy revealed that some species are hybrid between annual and perennial forms (such as *H. micranthus*) and enabled us to propose three genomes.

Both correspondence analysis and phenograms clearly delimited the 3 sections because most of markers were specific to sections. Moreover, the structure of the data was so strong that all the methods - even those less adapted to such data - led to the same partition in sections. Phenograms enabled to us to group species, but the comparison with taxonomy according to series was difficult due to the poor sampling of our species. Only 40 species were available in Montpellier.

Helianthus are native of 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.

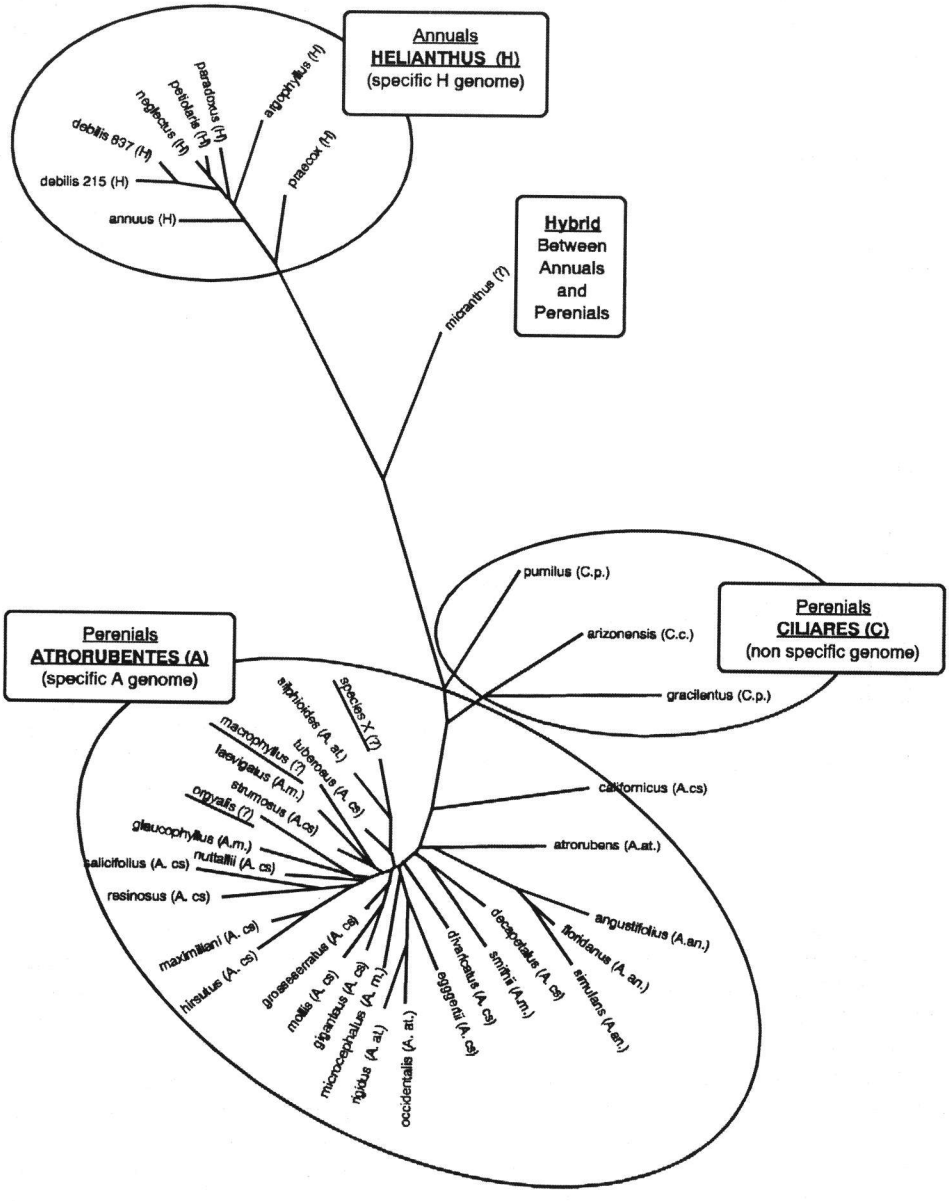


Figure 4: Phenogram of the 36 classified species using the Sokal and Michener dissimilarity with SCO-matrix and the NJ algorithm.

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 fit between the taxonomy of the genus and the classification of species on the basis of RAPD fragment distribution suggested the presence of 3 different genomes. These genomes were also found in related genera belonging to the *Helianthinae* subtribe.

Our method for screening primers appeared 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 have led to several hundred fragment positions. The characterization of the fragments was not realistic. Most of the fragments are expected to be polymorphic between plants of one species, while those conserved for a section should be present in all plants and without any polymorphism of one species belonging to this section, this was verified across experiments. Consequently, the choice of only one individual per species is justified as the chosen individual has no consequence on the score of fragments. Furthermore, we observed that closely related species frequently displayed fragments of the same size in comparison with 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 is 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 one probe hybridized not only the visible fragments but also undetected fragments, we studied a new score matrix (SCO) combiningthe GEL and the specific information from the HYB scores. The two matrices contain 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 leading to several hybridization signals.

The four phenograms (Figure 4) display groups of species corresponding to the three sections and to the four *Atrorubentes* series. Moreover, the relationships between series corresponded to those proposed by Schilling and Heiser (1981). The *Corona-Solis* and the *Microcephali* are closer than *Atrorubentes* and the *Angustifolii*. The relationships between species in each series suffer from the low number of fragments chosen to construct the series. Our knowledge of the intraspecific variability for any *Helianthus* species would infer that a larger sample of populations would be needed to establish the correct relationships between species. However, the RAPD fragments clearly enabled us to separate the *Corona-Solis* into three groups of 6, 5 and 3 species, although it did not coincide with the current taxonomy.

Table 3: Taxonomy of the second set of *Helianthus* species and related genera of *Helianthinae*

Lane number	Genus	Section	Species	*n*
1	*Helianthus*	*Helianthus*	*annuus*	17
2			*debilis tardiflorus*	17
3			*argophyllus*	17
4			*praecox*	17
5			*neglectus*	17
6			*petiolaris*	17
7			*paradoxus*	17
39			*niveus*	17
19			*micranthus*	17
27		*Agrestes*	*agrestis*	17
8 & 37		*Ciliares*	*ciliaris*	51?
9			*arizonensis*	17
10			*gracilentus*	17
11			*pumilus*	17
12		*Atrorubentes*	*mollis*	17
13			*resinosus*	51
14			*divaricatus*	17
15			*hirsutus*	34
16			*strumosus*	51
17			*rigidus*	51
35			*porteri*	17
18			*tuberosus*	51
38			*angustifolius*	17
20			X	?
21	*Viguiera*	sub-genera *Bahiopsis*	*tomentosa*	18
22	*Viguiera*	*Maculatae*	*dentata*	17
23	*Viguiera*	*Maculatae*	*eriophora*	17
31	*Viguiera*	*Diplotischis*	*quitensis*	17
24	*Tithonia*		*rotundifolia*	17
25	*Simsia*		*calva*	17
26	*Pappobolus*		*imbaburensis*	17
28	*Lagascea*		*helianthoides*	17
29	*Alvordia*		*brandegei*	17
30	*Heliomeris*		*multiflora*	8
32	*Iostephane*		*heterophylla*	?
33	*Rhysolepsis*		*palmeri*	?
34	*Almada*		*dentata*	?
36	*Phoebanthus*		*grandiflora*	17
40	*Flourensia*		*cernua*	?

Table 4: Classification of forty *Helianthus* taxa on the basis of the RAPD fragments

Section	Series	Species	New classified
Helianthus		*H. annuus*	
		H. debilis	
		H. petiolaris	
		H. paradoxus	
		H. argophyllus	
		H. praecox	
		H. neglectus	
		H. niveus	
hybrid species			*H. micranthus*
Atrorubentes	*Divaricati*	*H. divaricatus*	
		H. decapetalus	
		H. eggertii	
		H. californicus	
			H. macrophyllus
	Gigantei	*H. grosseserratus*	
		H. tuberosus	
		H. strumosus	
		H. giganteus	
		H. nuttallii	
		H. mollis	
	Corona-Solis	*H. hirsutus*	
		H. maximiliani	
		H. resinosus	
	Microcephali	*H. microcephalus*	
		H. laevigatus	
		H. glaucophyllus	
		H. smithii	
		H. silphioides	
			H. orgyalis
			H. sp
	Atrorubentes	*H. atrorubens*	
		H. occidentalis	
		H. rigidus	
		H. salicifolius	
	Angustifolii	*H. angustifolius*	
		H. floridanus	
		H. simulans	
Ciliares		*H. arizonensis*	
		H. gracilentus	
		H. pumilus	

The most parsimonious trees obtained with the SCO matrix were computed with either *H. annuus*, *H. mollis* or *H. gracilentus* as an outgroup. The most parsimonious trees of each combination were studied (not shown). They all displayed the three sections. However, the branches of these phylogenetic trees do not correspond to the series. Two possibilities may partially explain the discrepancies:

- the screening was too limited to reveal suitable fragments for recognizing the series;
- the parsimonial analysis may not be adapted to RAPD data.

The differences observed between the phylograms and the phenograms are, in all likelihood, due to the lack of knowledge of the homologies between the scores noted 1 and those noted 0.

The 3 main sub-groups of *Helianthus* correspond to the 3 sections because *H. agrestis* was not available. Whatever the combination score / genetic distance / tree algorithm, the phenograms display the three sections. Moreover, the phylograms also display the 3 sections. The advantages of the method by which these sections *Helianthus*, *Atrorubentes* and *Ciliares* were built up are that they were defined not only statistically, but also by 49 common fragments, whereas 29 and 17 fragments characterize the *Helianthus* and the *Atrorubentes*, respectively. Consequently the species are directly assigned to the sections and moreover any plant resulting from inter-section hybridization will be detected without ambiguity. The two genetic distances differ by the weight given to the common absence of fragments. Peltier *et al.* (1995) have proposed that JD is more adapted for distant member relationships because there is no allelism inference between common absence of any pair, whereas with SMD there is an allelism inference.

The main fact emerging from this study is dealing with the RAPD fragments of the same size and sharing a strong homology which appeared as key fragments in the molecular classification. Recent studies carried out with the RAPD cloned fragments have shown that they corresponded to repeated sequences. RAPD fragments have been reported as being efficient for *Petunia* taxonomy (Peltier *et al.*, 1995); here the division into sections is clearly revealed and, furthermore, *P. hybrida* still carries unrearranged chromosomes from ancestral species (Peltier *et al.*, 1994). We have no hypothesis to explain such conserved RAPD fragments since highly repeated sequences which usually evolve rapidly. These fragments are of great interest in botany for taxonomy and in genetics to detect hybrid forms. Moreover, they provide an opportunity to look at the related genera, *Viguiera, Phoebanthus, Heliomeris etc.*, of the *Helianthinae* sub-tribe. Most of these fragments have been cloned. They will be used for *in situ* hybridization and will be sequenced to look at their distribution and homologies.

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RELACIONES MOLECULARES DEL GENERO *Helianthus* A BASE DE RAPD MARCADORES

RESUMEN

Las relaciones feneticas y filogeneticas del genero *Helianthus* han sido determinadas a base de los marcadores moleculares. Cuarenta taxones de 36 especies y 4 numeros no clasificados en la coleccion de *Helianthus* han sido investigados por la utilizacion de RAPD tecnologia. 10 mer primers fueron investigados con respecto a los fragmentos intensificantes comunes para las especies que pertenecen a una seccion o que son comunes para todas especies dentro del genero. La homologia de secuencias entre RAPD fragmentos de misma grandeza fue confirmada por la hibridizacion molecular. Para la mayor parte de fragmentos fue constatado que ellos eran igualmente grandes en todas especies investigadas y que ellos eran homologos tanto como lo indico la hibridizacion molecular. De 118 fragmentos observados, 33 eran comunes para todas especies en el genero de *Helianthus*, 56 eran especificos para las especies de varios años, de los cuales 24 eran especificos para la seccion *Atrorubentes*, 29 para la seccion *Helianthus*, mientras la seccion *Ciliares* no tenia fragmentos especificos. La presencia o ausencia de RAPD (fragmentos o hibridizaciones señales) fueron utilizadas para la determinacion de todas estructuras por medio del analisis de correspondencia, con el fin de calcular las distancias geneticas. El analisis de correspondencia distinguio claramente las tres secciones antes mencionadas y señalo algunas series. La distancia de Jaccard y la semejanza de Sokal y Michener eran utilizadas dentro del metodo UPGMA y del metodo de conexion de vecinos para construir los dendogramas feneticos semejantes con esos que sostiene la taxonomia actualmente aceptada. La simpleza de la caracterizacion de fragmentos indica que el metodo RAPD es el ayudo importante para las investigaciones taxonomicas.

RELATIONS MOLÉCULAIRES DE L'*Helianthus* DÉTERMINÉES AU MOYEN DES MARQUEURS RAPD

RÉSUMÉ

Les relations phéniques et phylogéniques de l'*Helianthus* ont été estimées au moyen de marqueurs moléculaires. Quarante taxons appartenant à 36 espèces et 4 sortes non classifiées d'*Helianthus* ont été examinées à l'aide de la technologie RAPD. Les fragments amplifiants de 10 mer primers particuliers communs à l'espèce appartenant à une section ou communs à toutes les espèces du gène ont été examinés. L'homologie de la séquence entre les fragments RAPD de même dimension a été confirmée par hybridation moléculaire. Des 118 fragments retenus, 33 étaient communs à toutes les espèces d'*Helianthus*, 56 étaient particuliers à des espèces vivaces. Parmi eux, 24 étaient particuliers à la section *Atrorubentes*, 29 à la section *Helianthus*, et aucun à la section *Ciliares*. La présence ou l'absence de RAPD (fragments ou hybridations signalétiques) ont été utilisées pour déterminer toutes les structures au moyen de l'analyse de la correspondance et dans le but d'évaluer les distances génétiques. L'analyse de la correspondance a clairement distingué les trois sections et identifié quelques séries. La distance Jaccard et la similitude Sokal et Michener ont été choisies avec la méthode UPGMA et la méthode de liaison des voisins pour construire des arbres phéniques qui étaient très similaires à ceux de la taxonomie actuelle. La simplicite de la caractérisation des fragments montre que la méthode RAPD est une aide importante dans les recherches en taxonomie.