RAPD markers specific to sections in *Helianthus* reveal ancient genome and allopolyploid and autopolyploid origins in the genus

Khalid Sossey-Alaoui[1], Edward Schilling[2], Hervé Serieys[3], Michel Tersac[1], Patrick Lambert[1] and André Berville[1$]

INRA-Montpellier, Station d'Amélioration des Plantes, Laboratoire du Tournesol,
[1]2, Place Pierre Viala, F-34060 MONTPELLIER CEDEX 1
[3]Domaine de Melgueil F-34130 MAUGUIO CEDEX
[2]University of Tenessee, Department of Botany, Knoxville TN 37996-1100, USA.
[$]To whom correspondence should be sent : Tel 33 67 61 22 33 and Fax 33 67 04 54 15
E-mail: berville@msdos.montpellier.inra.fr

Summary: Ten mer primers were screened among those amplifying common fragments to *Helianthus* species belonging to one section or to the whole genus. Fragments of the same size from one primer amplified either in sunflower (*H. annuus*) or in Jerusalem artichoke (*H. tuberosus*) were conserved in other species in size and still shared homology as judged by molecular hybridization. Out of 88 fragments from 21 primers, 16 were common to *Helianthus* species, 20 were unique to sect. *Atrorubens*, 13 were unique to sect. *Helianthus*, whereas 0 were unique to sect. *Ciliares*. Each set of fragments was assumed belonging to one genome, (1) the C genome carrying the fragments common to all species of the 3 sections, (2) the H genome unique to sect. *Helianthus* and the D genome unique to sect. *Atrorubens*. Homologies were revealed in 3 genera out of 15 of the *Helianthineae* sub-tribe by molecular hybridization of the amplification products. The simple way used to characterize these fragments led to powerful tools to recognize different genomes for genera which may have diverged 1-20 millions years ago. This method would therefore enable to establish species or genera relationships even without knowledge *a priori*.

The taxonomy of the *Helianthus* genus has been frequently revisited until Schilling and Heiser (1981) have proposed four sections: *Helianthus*, *Atrorubens*, *Ciliares* and *Agrestes*, of which some are further divided into series. The species carry at least 34 chromosomes (n=17) and are considered as diploid, tetraploid (n=34) or hexaploid (n=51). In contrast, the basic chromosome numbers in the *Helianthineae* sub-tribe are 8, 17 and 18, which argues against a likely amphiploid origin for a 17 chromosome set as has been suggested by Heiser and Smith (1955). Moreover, for *Viguiera* the 2 basic numbers of o chromosomes 18 and 17 correspond to primitive and specialized species, respectively (Do you have reference for that?)

We used here the fragments generated by ten mer primers (RAPDs) as molecular markers. Primers were screened (35/100) for amplified fragments common for several species. We found that 35 out of 100 primers assayed led to such fragments. Surprisingly we found that mostly of the fragments were either specific to one section or common to all the species of the 3 sections (Sossey-Alaoui *et al.*, 1993). Twenty-one primers were used onto DNAs from a set of thirty-five species belonging to sect. *Helianthus*, sect. *Atrorubens* and sect. *Ciliares*. They enabled the amplification of 88 fragments. Among these fragments 16 were common to all species of *Helianthus*, twenty were unique to sect. *Atrorubens*, thirteen were unique to sect. *Helianthus* whereas none (0) were unique to sect. *Ciliares*. The fragments are conserved in size and are still homologous as judged by molecular hybridisation of Southern gel transfers with the reference fragments from sunflower (sect. *Helianthus, H. annuus*) or from Jerusalem artichoke (sect. *Atrorubens, H. tuberosus*), as a probe. We assumed that each specific set of fragments belong to three different genomes, (1) the C genome present in the three sections, (2) the H genome unique to the sect. *Helianthus* and the D genome unique to the sect. *Atrorubens*.

Then we looked for these fragments in 15 related genera of the *Helianthineae* and few other *Helianthus* species using molecular hybridization as previously described. We first verified that fragments unique to species of the sect. *Helianthus* were also present in the new sect. *Helianthus* specimen. Second, we detected signals in *H. agrestis* (sect. *Agrestes*) and in *H. porteri* (the only annual species belonging to the sect. *Atrorubens*) corresponding to a different size but common for the 2 species.

Hybridization signals with the amplification products from *Helianthus* and the sunflower reference fragments indicated that there are a common genome between theses species. In the same way signals with the amplification products from the sect. *Atrorubens* species and the *H. tuberosus* reference fragments indicated another genome these species. The three sect. *Ciliares* did not display any of the fragments unique to either the sect. *Helianthus* or the sect. *Atrorubens*. The sect. *Ciliares* did not display any specific fragment. In comparison with the two other sections this lack of fragment cannot be due to chance. Moreover, we detected fragments which are always present whatever the species studied. The amplified fragments (88) from the set of primers (21) show a distribution among 35 species which enables to us to recognize that most of them are related to the taxonomic sections. This correspondence cannot be due to chance since it occured for 49 fragments, out of 88, which can be considered to be widespread in the genomes of the three sections. Consequently, the genomic structure of the two sections should be: - HC for the sect. *Helianthus*, - DC for the sect. *Atrorubens*. These two sections like the sect. *Ciliares* carry a multiple of $n = 17$. The diploid sect. *Ciliares* must therefore to carry two C genomes (CC).

The polyploidy level seen today in the sect. *Atrorubens* and the sect. *Ciliares* is most likely due to duplicate or triplicate genomic stocks, DC and CC, respectively. Several facts sustain our model of allopolyploidy or autopolyploidy in *Helianthus*. A possible polyploid origin of $n=17$ for *Helianthus* has been proposed by Kostoff (1939) has recognized 2 genomes in *H. tuberosus* (2n=6x) and we found that it carries DDDCCC. Efremov (1967) has observed at meiosis tetravalents and tetravalent chains in the tetraploid sunflower. Allopolyploidy has already been suspected because of chromosome pairing in haploids or aneuploids and because multivalents occured during meiosis in *H. petiolaris*, *H. annuus*, their hybrids and in different progenies of sect. *Ciliares* from anther culture (Asad, 1985; Jackson & Murray, 1983). Anisimova (1984) has suggested a more detailed model for genomic organization in *Helianthus* studying storage protein and has suggested relationships of species in concordance with helianthinin patterns (Anisimova *et al.*, 1993)which are in agreement with our model. Moreover the cDNA probes which were screened to construct a genetic RFLP map have been found repeated in at least two copies (70 %) and more than two copies. These copies are not in a cluster but are present in different linkage

groups (Gentzbittel *et al.*, 1994b, 1995). The simple way to display two sets of cDNA is by old polyploidisation. The facts which argue against our presentation are that several phylogenies based upon the chloroplast DNA, on the ribosomal DNA or on RFLP do not involve amphiploidisation (Rieseberg *et al.*, 1991; Rieseberg, 1991, Gentzbittel *et al.*, 1992). However, both markers cannot reveal all the history of species since the cp DNA is inherited maternally and the rDNA represent few loci (most likely 3).

Our model of allopolyploidisation in *Helianthus* and in the other related genera *Viguiera*, *Phoebanthus* and *Pappobolus* is simple. Now, we have molecular tools represented by numerous RAPD fragments, of which some have already been cloned, in order to search for homologies in other genera related to *Helianthus*. This method to screen fragments unique to taxonomic divisions in *Helianthus* might be extended to look for any genetic relationships in many complex genera.

Acknowledgements: This work was supported partly by CETIOM and PROMOSOL.

LEGENDS FOR FIGURES

Figure 1: Amplification products with the OPC02 primer separated on 2.2% agarose gel and stained with etidium bromide. Lanes 1-40 correspond to 40 samples representing 40 *Helianthus* species

Figure 2: Southern transfer hybridised with the OPC02-1,400 fragment as a probe from *H annuus*.

Figure 3: Southern transfer hybridised with the OPC02-1,200 fragment as a probe from *H. tuberosus*.

REFERENCES

Anisimova, I.N. (1984).Genetica 20: 1925-1933 (in Russian).

Anisimova, I.N., Georgieva-Todorova, J., & Vassileva R. 1993. Helia 16, 14, 18: 49-58:

Asad, A. (1985). Thèse Université de Montpellier II, 198pp.

Efremov, A.E. (1967). Genetica 11: 31-36 (In Russian).

Gentzbittel, L., Perrault, A, & Nicolas, P. (1992). Mol. Biol. Evol. 9: 872-892.

Gentzbittel, L., Zhang, G., Vear, F., Griveau, Y., & Nicolas, P. (1994a). Theor Appl Genet 89:

Gentzbittel, L., Zhang, G., Vear, F., Bervillé, A., & Nicolas, P. (1995).Theor. Appl. Genet. (in press).

Heiser, C.B., & Smith, J.D.M., (1965). Proc. Ind. Acad Sci. 64: 250-253.

Heiser, C.B., & Smith, J.D.M., Clevenger, S.B., & Martin, W.C. (1969). Memoirs of the Torrey Botanical Club 22: 1-218, 5.

Jackson, R.C., & Murray, B.G. (1983). Theor. Appl. Genet. 64: 219-22.

Kostoff, D. (1939). Genetica 11: 258-300 (In Russian).

Peltier, D., Farcy, E., Dulieu, H., & Bervillé, A. (1994). Theor. Appl. Genet. 88: 637-645.

Rieseberg, L.H., (1991). Evolution 78: 1218-1237.

Rieseberg, L.H., Beckstrom-Sternberg, S., Liston, A., & Arias, D.M. (1991). Syst Bot 16: 50-76

Schilling, E.E., & Heiser, C.B. (1981). Taxon 30: 393-403

Sossey-Alaoui, K., Serieys, H., Lambert, P., Zaharieva, M., Peltier, D., Belhassen, E., & Bervillé, A. (1993). Biotechnology and Biotechnological Equipment, series B (Sofia), 7 (4): 21-31.

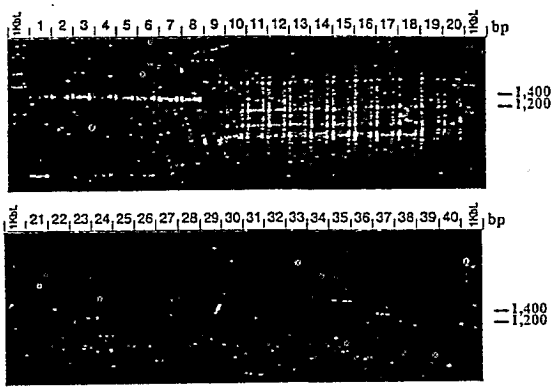

Fig. 1

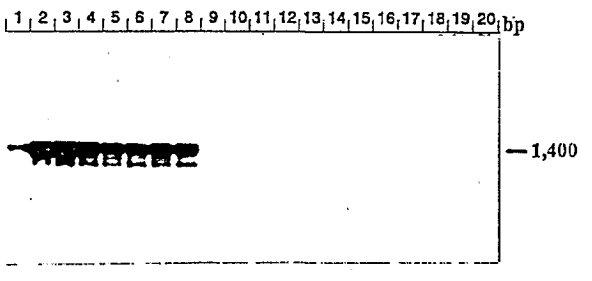

Fig. 2

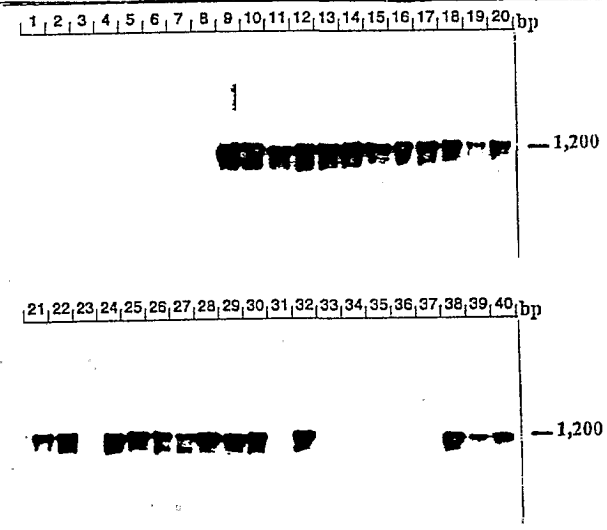

Fig. 3