Genetic Mapping of an Intergeneric Citrus Hybrid Using Molecular Markers

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A Citrus genetic linkage map was constructed: it can be used in interspecific and intergenetic hybrid heredity studies and for determining genes of agronomic interest.

introduction

Many problems are encountered when conducting genetic analyses of citrus hybrids, mainly for the following reasons: (1) high heterozygosity, (2) partial apogamy (polyembryonic seeds with embryos of nucellar origin and an embryo of zygotic origin), (3) the high numbers of progenies, and (4) the long juvenile phase (Gmitter *et al.*, 1992). The genetic improvement potential of cultivated citrus has been enhanced through biotechnological advances and the development of molecular marker techniques. Gene mapping has been carried out by several laboratories (Durham *et al.*, 1992; Jarrel *et al.*, 1992).

A citrus genetic linkage map was constructed using different types of molecular markers (isoenzymes, RFLP and RAPD), and the main applications are:
– investigate chromosomal heredity in interspecific and intergeneric hybrids,
– determine the number and assess the impact of detrimental or unsuitable mutations when crossing polyembryonic species,
– locate genes involved in the development of agronomically interesting characters. Markers associated with these genes could then be used for early selection of progenies and possible progenitors for breeding programmes (de Vienne, 1984; Stuber, 1989). Moreover, markers could potentially be used to isolate and transfer genes by genetic engineering (Ganal *et al.*, 1991).

A linkage map of a citrus genome from a hybrid obtained by a three-way cross is presented, focusing specifically on segregation distortions. The different analytical procedures are discussed in terms of the underlying biological models.

material and methods

hybridization

The progeny investigated (52 plants) were obtained by crossing a *Citrus grandis* (cv Seedless pummelo) female parent with an intergeneric hybrid male parent. The later was obtained through a *Citrus reshni* Hort. ex Tan. (cv Cleopatra mandarin) x *Poncirus trifoliata* L. Raf. (cv Swingle) cross.

markers

About one hundred markers were obtained. About half of these were RFLP markers from two cDNA banks (Luro, 1993). Forty probes were derived from a cDNA bank that we obtained from cv Valencia Late orange (*Citrus sinensis*) mRNA. Four other probes from a *Citrus jambhiri* bank were supplied by Dr. Roose of Riverside University, California (Jarrell *et al.*, 1992). Only cDNas with slightly repeated sequences or unique sequences showing polymorphism between the parents used in the cross were considered.

About forty amplified fragments segregating in the progeny were selected with a system involving RAPD markers (Williams *et al.*, 1990) using operon primers. Selection was based on the repetability of the amplifications and intensities of the amplified fragments. Heredity was also studied in a few fragments amplified by primers representing microsatellite sequences ([TCC]5; [GACA]4).

Seven isoenzyme systems were utilized: isocitrate dehydrogenase (ICD), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), endopeptidase (GOT) and phosphoglucomutase (PGM). Three isoenzymatic loci (LAP, GOT and PGI) were heterozygotic in pummelo and six (End, PGM2, LAP, GOT, ICD and MDH1) in the intergeneric hybrid (male).

genetic mapping

The genetic linkage maps were constructed using the Mapmaker software package (LANDER *et al.*, 1987). Several *Lod score* (logarithm of odds ratios) thresholds were tested to construct linkage groups. HALDANE's (1919) genetic mapping function was used. The recombination frequencies were calculated with the Mapmaker program.

The linkages and recombination frequencies (r) for each pair of markers were also calculated using the Genepop software program (OLLITRAULT, 1987). This program measures linkages by the chi-square test of independence (MATHER, 1957). This test takes segregation distortions into consideration relative to calculated theoretical frequencies for each class; it is based on marginal frequencies of the contingency table.

results and discussion

genetic linkage map

In all cases and for each individual hybrid, genetic compositions of male and female gametes for the intergeneric hybrid (*C. reshni* x *P. trifoliata*) were deduced from the genetic differences between parents used in the cross (Fig. 1). This progeny could thus be analysed for all markers as that of a test cross.

Forty-six fragments specific to the intergeneric hybrid (19 mandarin and 27 *Poncirus*) and 24 fragments specific to the maternal genome (pummelo) were amplified by 25 primers using the RAPD technique along with marker segregation in the progeny (Fig. 2).

The genetic linkage map constructed by the Mapmaker program included 95 markers distributed in 12 linkage groups (with a basic chromosome number of 9) (Fig. 3). Nine loci did not belong to any linkage group. The map was 1503 cM long (Haldane function), which is about two-thirds the total length of the genome (JARRELL *et al.*, 1992). The groups were established with a *Lod score* > 3 and maximum recombination frequency of 0.3 (corresponding to the approximate maximal detectable frequency for a backcross of 52 plants).

segregation distortions

Thirty-nine of the 104 markers mapped (37.5%) showed significant (chi^2 at 5% level) distortion as compared to the theoretical 1/1 segregation. In contrast, in pummelo, only 3 of 45 markers (6.5%) segregated in a non-Mendelian manner. Abnormal marker segregations have already been observed in citrus, especially in hybridizations of plants of different genera (TORRES *et al.*, 1985; OLLITRAULT & FAURE, 1992; DURHAM *et al.*, 1992; JARRELL *et al.*, 1992).

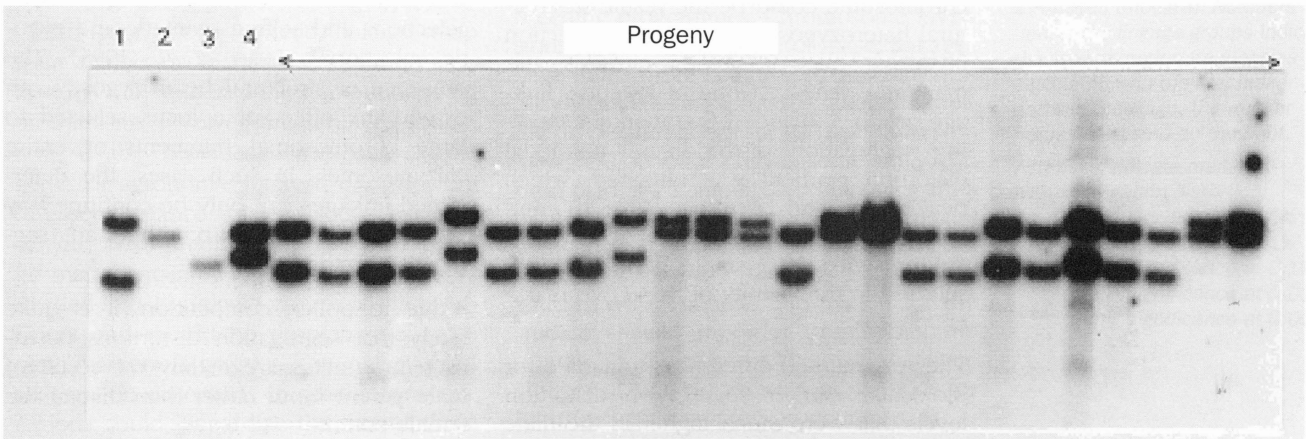

Figure 1
RFLP profile. Results of hybridization between DNA of parents and their progeny, digested by EcoRV and the cDNA probe 0.30.
1: cv Seedless pummelo
2: cv Cleopatra mandarin;
3: Poncirus trifoliata;
4: FAO 30573 (Cleopatra mandarin x Poncirus trifoliata*).*

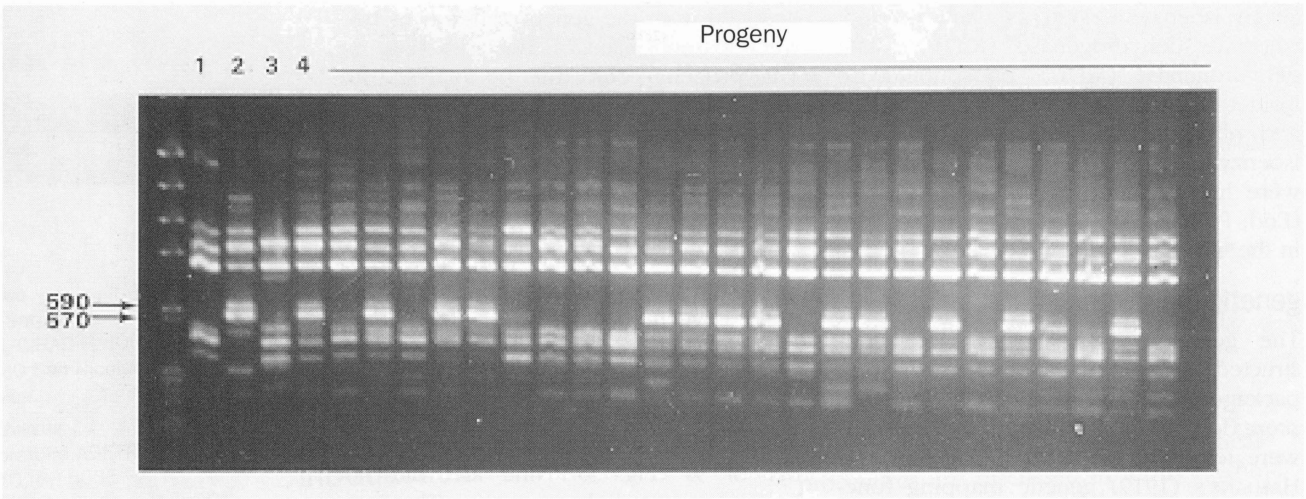

Figure 2
Segregation of RAPD markers. Results of amplification with primer OPK4, DNA from cv Seedless pummelo (1) x FAO 30573 (4) hybrids. The two 590 bp and 570 bp fragments were transmitted by cv Cleopatra mandarin (2). Lane 3: Poncirus trifoliata.

Note that linkage group 1 on the intergeneric hybrid genetic linkage map was constructed solely with markers showing segregation distortion, including almost half of all loci affected. Detailed analysis of distortions in linkage group 1 revealed that all of the markers had undergone selection in favour of the cv Cleopatra parent. Two hypotheses could be put forth:
– this is not a real group because the distortions caused false linkages;
– this group actually corresponds to a chromosome and thus the widespread distortion is probably partially due to structural heterozygosity of this chromosome set.

It is quite unlikely that there was a false linkage group since the chi^2 tests for independence, to assess possible linkages for each pair of markers, were always significant. Counter-selection of a whole chromosome could be the result of structural heterozygosity and gametic selection favouring one or several cv Cleopatra mandarin genes. At one of its ends, linkage group 3 included five markers showing segregation distortion. A unimodal distortion gradient was obtained, which peaked around markers VLc3.18 and VLc0.37. This pattern seems to indicate the presence of a single gene that was selected in the vicinity of these two markers.

The very marked differences, obtained for molecular marker segregation distortion levels that were much higher in the male intergeneric hybrid than in the female grapefruit parent, were in line with the biological data:

• data from the literature demonstrate that distortions are much more common in interspecific and intergeneric hybridizations than in intraspecific hybridizations (structural heterozygosity definitely has an important impact). The overall selection in linkage group 1 could be due to such structures;

• the two intergeneric hybrid parents of the intergeneric hybrid were polyembryonic whereas the pummelo was monoembryonic. It is therefore quite likely that the genetic load was lower in the pummelo parent. Indeed, facultative apogamy promotes buildup of mutations, in the heterozygotic state, that are detrimental or unsuitable in the homozygotic state. These mutations can induce abnormal segregations during crossing (gametic selection) and selfing (gametic and zygotic selections) (Luro *et al.*, 1995). The presence of structural heterozygosity can extend the effect of a locus selected to large chromosomal fragments or entire chromosomes. In such cases, the determined linkages can only be confirmed by comparisons with a map without any segregation distortions;

• due to pollen competition, it is quite likely that segregation distortions occurring in gametes are mainly the result of male parent input rather than that of the female parent.

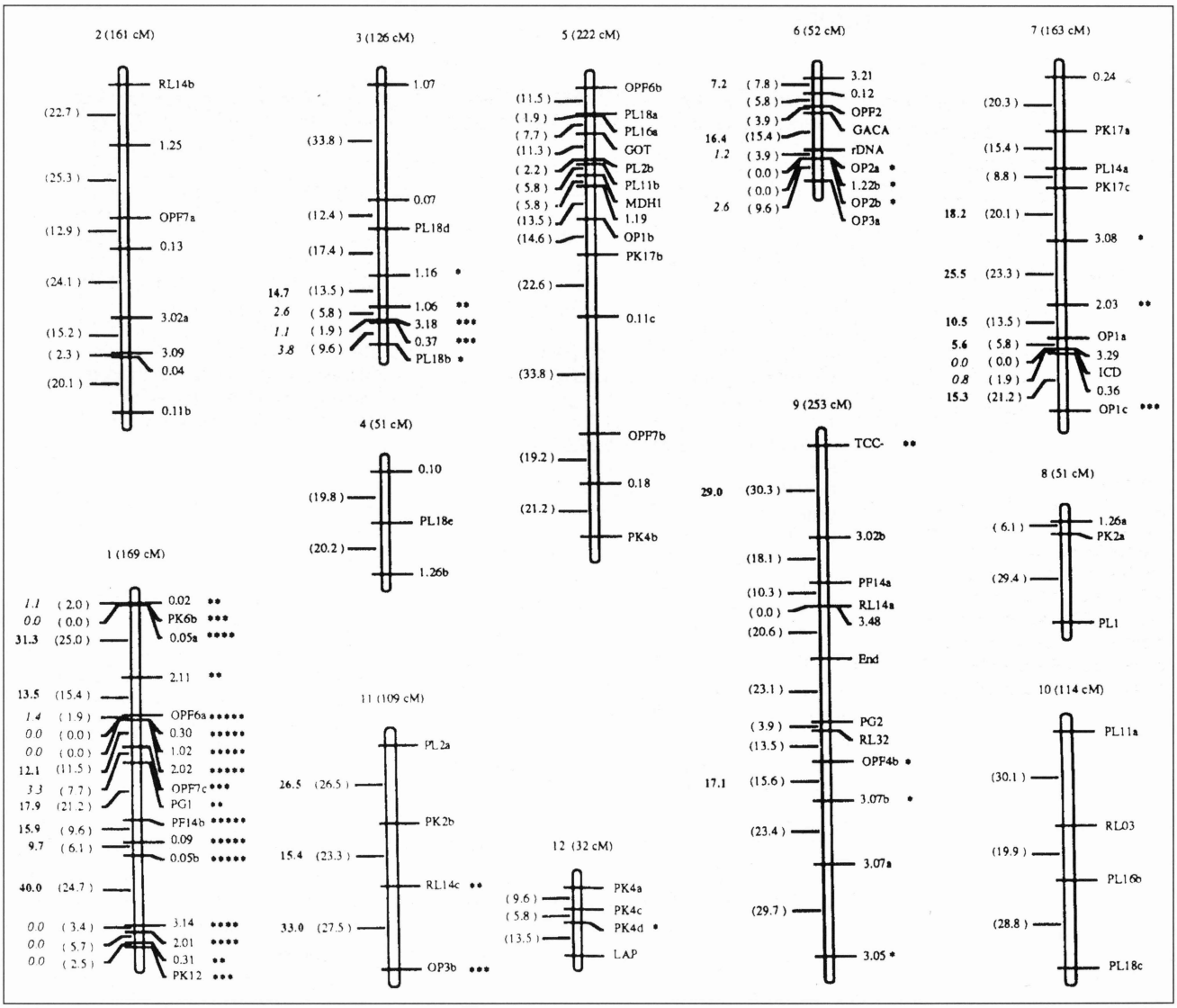

Figure 3
*Genetic linkage map for the intergeneric hybrid (*Citrus reshni *x* Poncirus trifoliata*). Numbers left of the linkage groups indicate the recombination frequencies calculated with the Mapmaker software package, the marker names are listed on the right.*

Asterisks indicate markers with segregation distortion:
**: chi² significance at 5%*
***: chi² significance at 1%*
****: chi² significance at 0.1%*
*****: chi² significance at 0.01%*
******: chi² significance at 0.001%.*

conclusion

Construction of a genetic linkage map from molecular markers is the first step towards fully understanding the underlying mechanisms involved in citrus reproduction. It also provides a means to locate genes of agronomic interest, e.g. showing disease resistance (tristeza, citrus bacterial canker, phytophtora, etc.). A partial genetic map containing one hundred markers divided into 12 linkage groups is still insufficient and should be supplemented with new markers. However, the presence of segregation distortions in specific genomic regions could jeopardize the use of these molecular markers for selection in plant breeding programmes. Chromosome inversions and the presence of false linkages have much more negative effects than simple distance estimate biases when attempting to locate genes or QTLs. Moreover, introgression of a chromosome fragment, promoted by a molecular marker with a distortion effect, leading to a false linkage with a sought-after trait, will result in breeding failures. It is thus essential to gain access to genetic information for the studied species in order to pinpoint the origins of distortions. This will facilitate choices of suitable assessment methods for future genetic mapping programmes.

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