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A kiwifruit (*Actinidia* spp.) linkage map based on microsatellites and integrated with AFLP markers

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Abstract A genetic map of kiwifruit (*Actinidia* spp.) was constructed using microsatellite and AFLP markers and the pseudo-testcross mapping strategy. $(AC)_n$ and $(AG)_n$ microsatellite repeats were first isolated from *Actinidia chinensis* (2n = 2x = 58) enriched genomic libraries and tested for segregation in the interspecific cross between the diploid distantly related species *A. chinensis* and *A. callosa*. Some 105 microsatellite loci of the 251 initially tested segregated in the progeny in a 1:1 ratio as in a classical backcross, or in a ratio which could mimic the backcross, and were mapped using 94 individuals. AFLP markers were then produced using *MseI* and *EcoRI* restriction enzymes and 15 primer combinations. Nearly 10% of *loci* showed a distorted segregation at $\alpha = 0.05$, and only 4% at $\alpha = 0.01$, irrespectively to the marker class. Two linkage maps were produced, one for each parent. The female map had 203 *loci*, of which 160 (71 SSR and 89 AFLP) constituted the framework map at a LOD score ≥ 2.0. The map was 1,758.5 cM(K) long, covering 46% of the estimated genome length. The male map had only 143 loci, of which 116 (28 SSR, 87 AFLP and the sex determinant) constituted the framework map. The map length was only 1,104.1 cM(K), covering 34% of the estimate genome length. Only 35 SSR *loci* were mapped in the male parent because 18% of SSR loci that were characterised did not amplify in *A. callosa*, and 48% were homozygous. The choice of parents in the pseudo-testcross is critically discussed. The sex determinant was mapped in *A. callosa*.

Keywords Molecular markers · Linkage map · Pseudo-testcross · Mutation · Sex marker

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Introduction

Seeds of *Actinidia deliciosa* [(A. Chev.) C.F. Liang et A.R. Ferguson] were introduced from China, the home of the genus *Actinidia*, to New Zealand at the beginning of the 20th century, and a small number of male and female plants selected from those seedlings gave rise to the few cultivars upon which the world-wide kiwifruit industry currently relies (Ferguson et al. 1996). Today, kiwifruit are considered an important fruit crop of temperate regions and are planted world-wide. The genetic base of the kiwifruit industry is extraordinarily narrow in comparison with the vastness of the wild genetic resources (Ferguson et al. 1996). The genus *Actinidia* includes more than 60 species, many of which have edible fruit (Liang 1984).

Kiwifruit have so far received very little attention from geneticists or breeders. One reason is that kiwifruit are such a new crop; another is that *A. deliciosa* is both dioecious and hexaploid ($2n = 6x = 174$), and these features make genetic studies difficult. *A. deliciosa* belongs to the *A. chinensis/A. deliciosa/A. setosa* complex, which encompasses diploid, tetraploid and hexaploid races with morphological traits so similar that they have been treated, until recently, as a single species by botanists (Liang 1984). The polyploid races of the complex may be derived from diploid *A. chinensis* genotypes without there being a contribution from any other *Actinidia* species (Testolin and Ferguson 1997). This suggests that if genetic studies are carried out on diploid races of *A. chinensis* the information obtained will be relevant to related polyploids such as tetraploid *A. chinensis* or hexaploid *A. deliciosa*.

Unfortunately, dioecy maintains high levels of heterozygosity in kiwifruit and precludes the possibility of producing inbred lines, which are the genotypes of choice for most genetic analyses, including linkage analysis. To overcome such a problem typical of allogamous species, a theoretical treatment has been developed for the estimation of recombination frequencies from populations obtained by crossing heterozygous

parents (Ritter et al. 1990; Ritter and Salamini 1996; Ridout et al. 1998). The mapping populations produced using such a pseudo-testcross (Grattapaglia and Sederoff 1994; Hemmat et al. 1994) are becoming fairly common for the construction of linkage maps in outcrossing species. We adopted such a strategy for obtaining a linkage map in diploid races of *Actinidia*. We started by sequencing and mapping microsatellite *loci* (Huang et al. 1998).Then, we adopted the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) for the rapid production of further markers to saturate the linkage groups.

Materials and methods

Plant material

A mapping population was obtained by crossing a diploid female genotype (no. 54.20) of *A. chinensis* (2n = 2*x* = 58) with a diploid male genotype (no. 103) of *A. callosa* Lindl. var. *henry* Maxim. (2n = 2*x* = 58), a distantly related species.

Total DNA was extracted from 94 individuals as well as from the parents using 1 g of young developing leaves and the procedure described by Doyle and Doyle (1990), as modified slightly by Cipriani and Morgante (1993). DNA was then purified of polysaccharides with 2-butoxyethanol according to the procedure described by Manning (1991).

Microsatellite markers

Details of the procedures have already been published (Huang et al. 1998). Genomic DNA from diploid *A. chinensis* no. 54.20 was digested with *Tsp509I*, and fragments in the size range 200–800 bp were hybridised to biotinylated $(GT)_{13}$ or $(CT)_{13}$ and selectively separated using streptavidin-coated paramagnetic beads (Dynabeads, Dynal, Oslo, Norway) to produce two genomic libraries enriched in $(AC/GT)_n$ or $(AG/CT)_n$ repeats, respectively, following the procedure reported by Morgante et al. (1998). DNA fragments recovered after the enrichment procedure were cloned into the *Lambda Zap II* vector (Stratagene) and used to transform XL1-Blue MRF *Escherichia coli* cells (Stratagene). Clones were screened for the presence of the desired repeat by plaque hybridisation with a digoxigenin-labelled probe. Positive clones were re-screened by means of the anchor polymerase chain reaction (PCR) (Rafalski et al. 1996), and those with the microsatellite misplaced were discarded. The remaining clones were sequenced.

Sequences were first compared with those already sequenced to avoid duplications. Primer pairs were designed for PCR in the microsatellite flanking regions using PRIMER SOFTWARE PACKAGE version 0.5, and later on version 3.0 (Whitehead Institute of Biochemical Research, Cambridge, Mass.) and assayed for segregation in both parents and a few progeny. Markers suitable for mapping (see below) were processed in the whole population.

PCR reactions were performed in a 25-µl volume containing 10 m*M* TRIS-HCl pH 9.0, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* each dNTP, 0.2 µM each primer, 100 ng of genomic DNA and 0.2 U of *Taq* polymerase (Pharmacia) using the following temperature profile: 95°C for 5 min, then 35 cycles of 94°C for 50 s, followed by 55°C for 50 s and 72°C for 50 s, finishing with 72°C for 8 min. PCR products were separated by electrophoresis on 3% Metaphor (FMC BioProducts) agarose gels and stained with ethidium bromide.

AFLP markers

To get a more saturated map, we also produced AFLP markers using the restriction enzymes *MseI* and *EcoRI* and 15 primer combinations, preliminarily selected from the parental DNA on the basis of the high number of fragments produced and their separation on acrylamide gel. DNA digestion, adaptor ligation and pre- and selective amplifications were carried out according to the manufacturer's instruction (GIBCO BRL, Life Technologies**)** and the standard procedures (Vos et al. 1995). Selective amplification was carried out using [^{33}P]-labelled *EcoRI* primer with the following temperature profile: 12 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s, with the annealing temperature lowered 0.7°C for each cycle (touch-down); 23 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 60 s. PCR reactions were mixed with 2.5 µl formamide dye, heated at 95°C for 5 min and then quickly cooled on ice. Three microliters of each sample was loaded on a 6% denaturing polyacrylamide gel (Long Range, FMC BioProducts) containing 7 *M* urea and run at 58 W for 2 h. After electrophoresis, the gel was dried on Watmann 3MM paper in a vacuum dryer at 80°C for 2 h and autoradiographed on X-ray film (Kodak) using standard procedures.

Sex determinant

Sex control in *Actinidia* is apparently monogenic with males being heterozygous at the sex-determining region (Testolin et al. 1995). Female and male segregated in our progeny accordingly (45:46 (male : female) ratio, 3 plants died before flowering), and the sex determinant was therefore included in the linkage analysis.

Linkage analysis

Data from segregation analysis were recorded into an electronic spreadsheet. Markers were grouped into three separated datasets according to their segregation pattern and the parent from which allele segregated as follows: (1) those which segregated from the mother as in a classical backcross ($ab \times n_1n_2$, where n_1n_2 could be any allele combination different from ab or ba); (2) those which segregated from the father as above; (3) those which segregate 1:2:1 or 3:1 as in a classical F_2 inter-cross. Co-dominant markers, with three or four alleles at stake, were considered to be two loci, one heterozygous in each parent. With this expedient, the segregation became of the type 1:1 and was scored as in a classical backcross. AFLPs, which are dominant markers, were included into the first or the second dataset according to the parent from which they segregated. Dataset (3) was later discarded because it included only one simple sequence repeat (SSR) marker.

Each of the first two datasets was duplicated and in the new matrix genotype coding was swapped (1 for 0 and 0 for 1). The original matrix and the new one were then merged. This enabled the determination of linkage between *loci* in mixed phases.

The linkage analysis was carried out using MAPMAKER/EXP 3.0 (Lander et al. 1987). The linkage groups were found with the GROUP command (LOD ≥ 4.0, maximum recombination fraction ≤ 0.3). The command produced two symmetrical sets of linkage groups, one of which was disregarded. Small linkage groups (≤ 8 markers) were then ordered with a multipoint exhaustive analysis using the COMPARE and MAP commands (LOD ≥ 2.0); larger groups were treated with the ORDER command (LOD ≥ 2.0). The RIPPLE function was employed at LOD ≥ 2.0 to assess the robustness of the marker order. Markers not placed in the framework map were finally added with the TRY command. These accessory markers do not contribute to the overall genetic distance of their linkage group. Map distances were calculated with the Kosambi function (Kosambi 1944). SSR loci segregating in a 1:1:1:1 ratio enabled anchor points to be established between homologous linkage groups in the two maps.

An estimate of genome length was finally carried out following method no. 3 of Chakravarti et al. (1991) using all loci and a lod threshold, Z = 3, 4 or 5.

Results

Marker production and testing for segregation

As a result of the enrichment procedure about 50% of the plaques were positive when hybridised with the probe complementary to the sought repeat (data not shown). Of the 529 positive clones, 54% were discarded in subsequent steps mainly because the repeat was too close to the cloning site (Table 1). A total of 233 microsatellites isolated in our laboratory were screened for segregation in the mapping population. A further 18 published by Weising et al. (1996) were also studied.

Only 102 primer pairs (41%) amplified loci that segregated in the progeny (Table 2). Four primer pairs amplified two polymorphic loci each, thus bringing to 106 the number of segregating SSR loci. Most of these loci (84%) segregated 1:1, 16 segregated 1:1:1:1 and only 1 segregated 1:2:1. We therefore decided to map using the backcross model of segregation and abandoned the microsatellite which segregated 1:2:1. Among loci suitable for mapping, 82% segregated from the female parent *A. chinensis*, from which microsatellites were isolated, and only 33% segregated from the male parent, *A. callosa* var. *henry* (Table 2). A mutation at the microsatellite locus UDK[99]-143 was noticed as a homozygous band from the male parent and was not amplified in one individual of the progeny, despite PCR being repeated several times.

We produced approximately 1,372 AFLP bands using 15 primer combinations (Table 3). Of these, 271 bands

Table 1 Efficiency of the procedure adopted for the isolation of microsatellite sequences in *Actinidia chinensis*

Step	*n*	Percentage[a]
Positive clones screened	529	–
Clones retained for sequencing after anchor PCR	341	64
Sequences containing the microsatellite repeat	328	62
Sequences suitable for primer design	244	46
Sequences correctly re-amplified in *A. chinensis*	244	46

[a] Calculated over the number of positive clones screened

Table 2 Summary of the SSR markers tested in the interspecific cross *A. chinensis* × *A. callosa* and type of segregation

	Total	*A. chinensis*	*A. callosa*
Number of primer pairs screened for segregation[a]	251		
Number of primers abandoned[b]	149		
Number of primer pairs analysed in the whole progeny	102		
Number of polymorphic loci[c]:	106		
segregating 1:1	89	70	19
segregating 1:1:1:1	16	16	16
segregating 1:2:1[d]	1		
Number of loci suitable for mapping	105	86	35

[a] A total of 233 isolated in our laboratory and 18 published in Weising et al. (1996)
[b] Forty-three gave no or weak amplification in parents; 49 were insufficiently resolved on agarose gel; 26 were apparently monomorphic on agarose gel; 31 did not segregate because two homozygous loci were likely amplified by the same primer pair
[c] Four primer pairs amplified two polymorphic loci each
[d] Abandoned because not suitable for the backcross model of segregation

Table 3 Summary of the AFLP markers segregating in the interspecific cross *A. chinensis* × *A. callosa*

Primer combination	Marker code	Total	*A. chinensis*	*A. callosa*
EcoRI-AAC / *MseI*-CAA	PK01-xx[a]	20	9	11
EcoRI-AAC / *MseI*-CAC	PK02-xx	23	8	15
EcoRI-AAC / *MseI*-CAT	PK03-xx	19	10	9
EcoRI-AAG / *MseI*-CTG	PK04-xx	25	16	9
EcoRI-AAG / *MseI*-CTT	PK05-xx	14	8	6
EcoRI-ACA / *MseI*-CTA	PK06-xx	19	10	9
EcoRI-ACA / *MseI*-CTC	PK07-xx	16	8	8
EcoRI-ACA / *MseI*-CTG	PK08-xx	21	6	15
EcoRI-ACA / *MseI*-CTT	PK09-xx	9	5	4
EcoRI-ACC / *MseI*-CTA	PK10-xx	14	7	7
EcoRI-ACC / *MseI*-CTC	PK11-xx	14	6	8
EcoRI-ACT / *MseI*-CAG	PK12-xx	22	12	10
EcoRI-ACT / *MseI*-CAT	PK13-xx	27	14	13
EcoRI-AGC / *MseI*-CTG	PK14-xx	22	14	8
EcoRI-AGC / *MseI*-CTT	PK15-xx	6	6	0
Total		271	139	132

[a] xx identifies the polymorphic fragments numbered progressively according to their size (the smaller the size the higher the number)

segregated 1:1 from the female or male parent in about the same proportion (Table 3). Nearly 10% of *loci* gave distorted segregation at $\alpha = 0.05$, and only 4% at $\alpha = 0.01$, irrespective of the marker class.

Linkage maps

The 226 loci segregating from *A. chinensis* (female parent) produced 38 linkage groups, of which 27 contained more than two *loci* (Fig. 1). As many as 160 *loci* (71 SSR and 89 AFLP) constituted the framework map

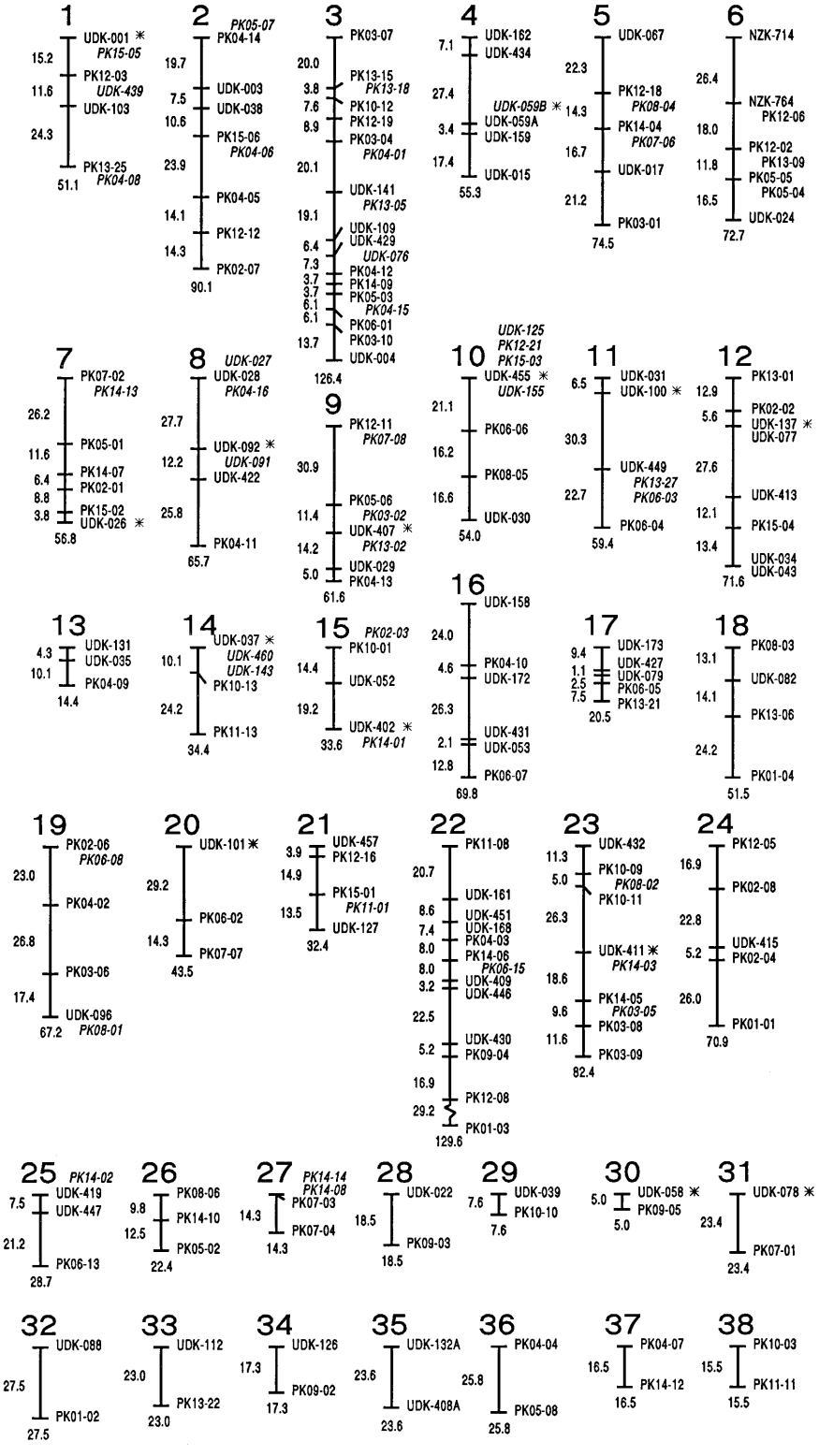

Fig. 1 Genetic linkage map of *Actinidia chinensis* (female parent). The *number* at the *top* of each linkage group has been arbitrarily assigned and corresponds to the linkage group with the same number in the male map only when the latter is not between brackets. Genetic distances in Kosambi centiMorgans are listed on the *left-hand side* and loci on the *right-hand side* of the linkage group. SSR loci are designated by *UDK-* xxx [*NZK*-designates those from Weising et al. (1996)], where xxx is a progressive code; AFLP loci by the *PK*xx- yy, where xx identifies the primer combination and yy identifies the polymorphic fragments within each primer combination numbered progressively according to their size (the smaller the size the higher the number). SSR markers followed by an *asterisk* are common to both maps. Framework markers, that could be ordered at a LOD score ≥ 2.0, are shown in *normal-type print*; accessory markers, that is markers that could not be ordered with equal confidence, are shown in *italics* in the most likely position in the framework map. The latter do not contribute to the total length of the linkage group, which is reported at the *bottom* of each drawing

at a LOD score ≥ 2.0, covering a genetic length of 1,758.5 cM (K). A further 43 *loci* (9 SSR and 34 AFLP) were included into linkage groups, but they could not be placed in the framework map and were therefore placed as ancillary markers. An additional 22 markers together with the sex determinant were found to be unlinked.

Only 168 loci segregated from *A. callosa*, the male parent, producing 30 linkage groups (Fig. 2). One hundred and sixteen loci contributed to the framework map (28 SSR, 87 AFLP and the sex determinant); 27 markers were placed in the map as ancillary markers (3 SSR and 24 AFLP); the remaining 25 markers were found to be unlinked. The total length of the map was 1,104.1 cM (K). The sex determinant was mapped in the male linkage group no. (25) (Fig. 2).

The expected *A. chinensis* map length, estimated following the method no. 3 of Chakravarti et al. (1991) and taking into account all *loci*, was 3,849 cM (3,969, 3,640, and 3,937 cM at a LOD threshold Z = 3, 4 and 5, respectively), whereas that of *A. callosa* was 3,199 cM (3,114, 3,234, and 3,250 cM at a LOD threshold Z = 3, 4 and 5 respectively). According to these estimates, the actual linkage maps should cover some 46% and 34% of the total genetic distance, respectively.

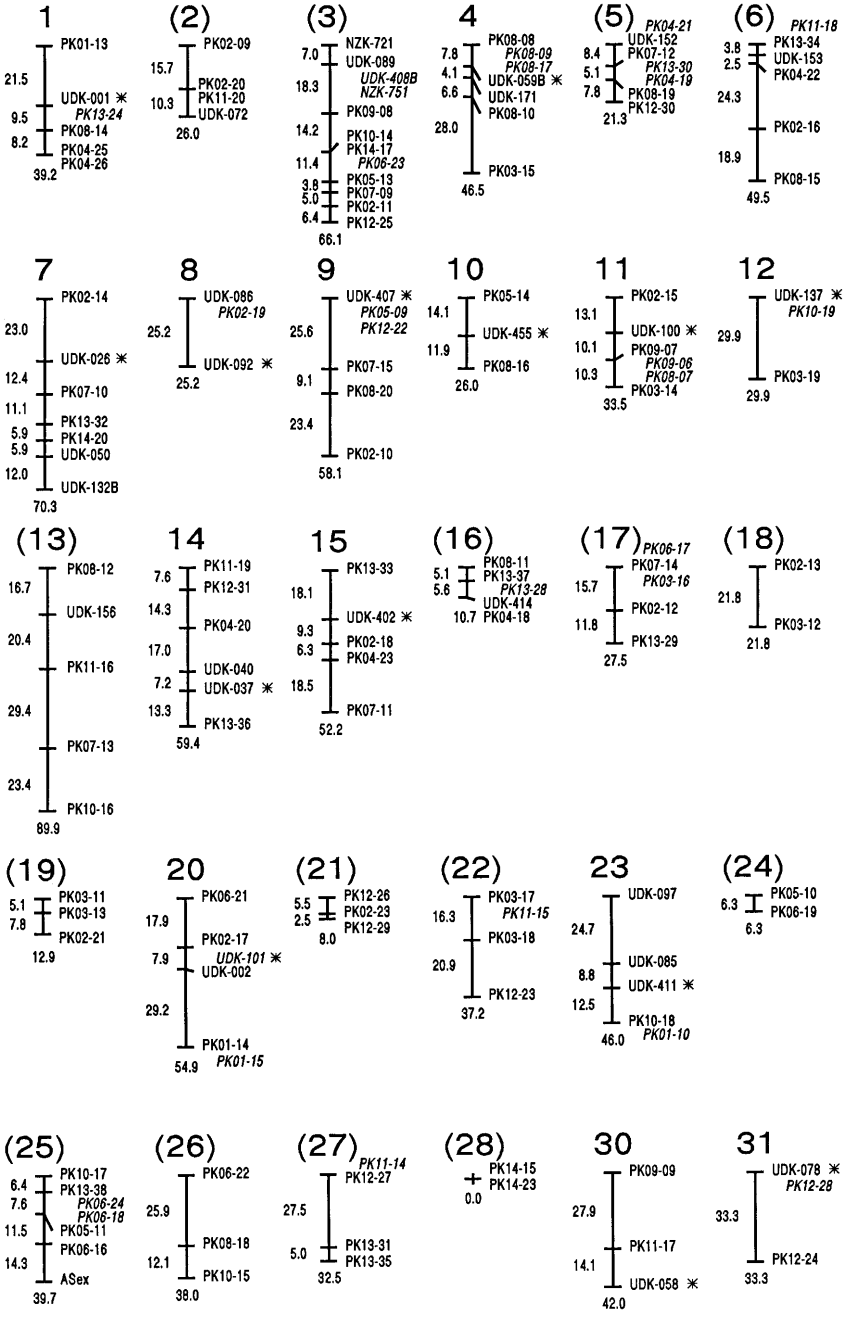

Fig. 2 Genetic linkage map of *Actinidia callosa* (male parent). The *number* at the *top* of each linkage group has been arbitrarily assigned and it corresponds to the linkage group with the same number in the female map only when it is not between brackets. SSR markers followed by an *asterisk* are common to both maps. See legend of Fig. 1 for other details

Discussion

Microsatellites proved to be very useful for mapping. The procedures used for library enrichment were very efficient with nearly half (46%) of positive plaques yielding a microsatellite sequence. If only the clones retained after anchor PCR are taken into account, the yield increases to 72%. Such an efficiency of library enrichment means that it can no longer be considered that the sequencing of microsatellites requires a large investment of time and effort. Furthermore, the high polymorphism and heterozygosity of microsatellites and their wide transportability across different mapping populations more than compensate for the effort involved in their isolation (Rafalski et al. 1996; Morgante et al. 1998).

We were able to map 41% of the microsatellites tested for segregation, but the percentage could have been higher had we used the better resolving power of acrylamide gels. Our success rates are similar to those reported for the only two available pseudo-testcross-based linkage maps in which an appreciable number of SSRs were placed: 40% for Norway spruce (Paglia et al. 1998) and 52% for avocado (Sharon et al. 1997).

Almost all SSR segregating loci (99%) segregated in a ratio suitable for mapping, as in a classical backcross, thus confirming that the pseudo-test-cross provides a very suitable strategy for mapping outcrossing species (Grattapaglia and Sederoff 1994; Hemmat et al. 1994). We used an interspecific cross between two taxonomically distantly related species (Liang 1984), with the aim of reducing the number of cases of common alleles in the two parents and the occurrence of segregation of the type 1:2:1, which could not be mixed with those typical of a backcross. We were indeed successful in avoiding such a risk, but there was an unforeseen negative feature of such a cross. Disappointingly, the number of SSR *loci* placed in the male map was significantly lower than those placed in the female one (35 vs 86). Nearly half the primers (48%) gave only a single band in the male parent and 18 (18%) yielded no amplification products. The lack of PCR products in the male parent was likely due to the wide taxonomic distance of *A. callosa* from *A. chinensis*, the female parent from which the microsatellites were isolated, whereas the low polymorphism of SSRs amplified in the male parent can be explained by the tendency of microsatellites to be shorter at homologous loci in species other than the one from which they were first isolated. This is certainly the case for *Actinidia* (Huang et al. 1998) and has also been reported for several plant and animal species (Ellegreen et al. 1995, 1997). The low number of SSRs placed in the male map made that map absolutely incomplete and reduced to only 14 the number of linkage groups for which the homology in the two maps was found.

Microsatellite loci were randomly distributed throughout the map. The 86 loci placed in the female map covered 33 of the 38 linkage groups, with only small groups of two and one of three markers lacking SSRs. The number of SSRs per group ranged from 0 to 6, with a limited clustering of these loci (Fig. 1). Similar results were found in the male map. These results are in agreement with the random distribution and the good genome coverage of microsatellite *loci* reported for the more highly saturated microsatellite-based linkage map of wheat (Roder et al. 1998).

The map produced for *A. chinensis* has only a moderate marker density, and it is not likely to be very useful in mapping or cloning genes of agronomic interest. It could be easily saturated further with AFLP markers, but we prefer in the future to continue isolating and mapping new SSRs. If we are able to produce a map backbone based on evenly spaced SSRs, we have a set of markers which can be easily transferred to virtually any new mapping population within the genus *Actinidia*. This should represent a robust tool for kiwifruit breeders aiming to develop marker-assisted selection and map-based gene cloning.

The sex determinant, which behaves like a monogenic Mendelian character, with the male sex being heterogametic (Testolin et al. 1995), was mapped only in the male linkage map, together with six AFLP markers but no SSR marker. We do not know whether the sex determinant was not placed in the female map because of a gap in that map or because of the low recombination of sexual chromosomes, as reported for animals and some dioecious plant species (Charlesworth and Guttman 1999). Since recombination in the sex-determining region has already been reported in *Actinidia* (Harvey et al. 1997), the first hypothesis is the more likely.

Several primer pairs amplified more than one locus. In a previous paper (Huang et al. 1998) we discussed the possibility that dipoid *Actinidia* species could themselves be polyploid in derivation. Two primer pairs amplified two loci, both of which segregated from the same parent. One pair [UDK(96)-020] gave two unlinked loci; the other [UDK(96)-059] amplified two loci that mapped very closely in the same linkage group (no. 4) (Fig. 1). It appears more likely that this is due to duplication of a DNA sequence rather than duplication of the whole genome. The evolution of microsatellites into more complex repeats through long-range slipstrand mispairing or unequal crossovers has been proposed by Stephan and Cho (1994, cited in Broun and Tanksley 1996). Further evidence is therefore required to establish whether genome duplication has occurred in diploid *A. chinensis*.

The estimate of genome length was 3,849 cM and 3,199 cM for *A. chinensis* and *A. callosa*, respectively. If the order of magnitude is correct, it appears the highest so far reported for a land plant. Among the list of species reported by Barreneche et al. (1998), there are three genera which have a genome length approaching 3,000 cM, namely *Malus*, *Populus* and *Vitis*. Of these at least *Malus* and *Vitis* are polyploid in derivation.

The mutation we found was probably due to a mutation at the 3' end, which reduced primer specificity and is likely the only scorable mutation among those which could occur in the SSR sequences we amplified. Considering that the map construction enabled the screening of

some 710,112 bases transferred from the parents to the offspring, a mutation rate of 0.71×10^{-6} can be estimated at the DNA sequence level per generation.

SSR primer sequences are available upon request free of any charge for non-profitable purposes.

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References

Barreneche T, Bodenes C, Lexer C, Trontin JF, Fluch S, Streiff R, Plomion C, Roussel G, Steinkellner H, Burg K, Favre JM, Glössl J, Kremer A (1998) A genetic linkage map of *Quercus robur* L. (pedunculate oak) based on RAPD, SCAR, microsatellite, minisatellite, isozyme and 5 S rDNA markers. Theor Appl Genet 97:1090–1103

Broun P, Tanksley SD (1996) Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol Gen Genet 250:39–49

Chakravarti A, Lasher LK, Reefer JE (1991) A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182

Charlesworth D, Guttman DS (1999) The evolution of dioecy and plant sex chromosome systems. In: Ainsworth CC (ed) Sex determination in plants. Bios, Oxford, pp 25–49

Cipriani G, Morgante M (1993) Evidence of chloroplast DNA variation in the genus *Actinidia* revealed by restriction analysis of PCR-amplified fragments. J Genet Breed 47:319–326

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Ellegren H, Primmer CR, Sheldon BC (1995) Microsatellite evolution – directionality or bias. Nat Genet 11:360–362

Ellegren H, Moore S, Robinson N, Byrne K, Ward W, Sheldon BC (1997) Microsatellite evolution – a reciprocal study of repeat lengths at homologous loci in cattle and sheep. Mol Biol Evol 14:854–860

Ferguson AR, Seal AG, McNeilage MA, Fraser LG, Harvey CF, Beatson RA (1996) Kiwifruit. In: Janick J, Moore JN (eds) Fruit breeding. vol 2. Vine and small Fruits crops. J Wiley & Sons, New York, pp 371–417

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121–1137

Harvey CF, Gill GP, Fraser LG, McNeilage MA (1997) Sex determination in *Actinidia*. 1. Sex-linked markers and progeny sex ratio in diploid *A. chinensis*. Sex Plant Reprod 10:149–154

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85:4–11

Huang WG, Cipriani G, Morgante M, Testolin R (1998) Microsatellite DNA in *Actinidia chinensis*: isolation, characterisation, and homology in related species. Theor Appl Genet 97: 1269–1278

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Liang CF (1984) *Actinidia*. In: Feng KM (ed) Flora reipublicae popularis sinicae, vol 49. Science Press, Beijing, pp 196–208, 309–324

Manning K (1991) Isolation of nucleic acids from plants by differential solvent precipitation. Anal Biochem 195:45–50

Morgante M, Pfeiffer A, Jurman I, Paglia G, Olivieri AM (1998) Isolation of microsatellite markers in plants. In: Karp A, Isaac PG, Ingram DS (eds) Molecular tools for screening biodiversity. Plant and animals. Chapman & Hall, London, pp 288–296

Paglia GP, Olivieri AM, Morgante M (1998) Towards second-generation STS (sequence-tagged sites) linkage maps in conifers: a genetic map of Norway spruce (*Picea abies* K.). Mol Gen Genet 258:466–478

Rafalski JA, Vogel JM, Morgante M, Powell W, Andre C, Tingey SV (1996) Generating and using DNA Markers in plants. In: Birren B, Lai E (eds) Nonmammalian genomic analysis. A practical guide. Academic Press, New York, pp 75–134

Ridout MS, Tong S, Vowden CJ, Tobutt KR (1998) Three-point linkage analysis in crosses of allogamous plant species. Genet Res 72:111–121

Ritter E, Salamini F (1996) The calculation of recombination frequencies in crosses of allogamous plant species with applications to linkage mapping. Genet Res 67:55–65

Ritter E, Gebhardt C, Salamini F (1990) Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. Genetics 125:645–654

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sharon D, Cregan PB, Mhameed S, Kusharska M, Hillel J, Lahav E, Lavi U (1997) An integrated genetic linkage map of avocado. Theor Appl Genet 95:911–921

Stephan W, Cho S (1994) Possible role of natural selection in the formation of tandem-repetitive noncoding DNA. Genetics 136:333–341

Testolin R, Ferguson AR (1997) Isozyme polymorphism in the genus *Actinidia* and the origin of the kiwifruit genome. Syst Bot 22:1–16

Testolin R, Cipriani G, Costa G (1995) Sex segregation ratio and gender expression in the genus *Actinidia*. Sex Plant Reprod 8:129–132

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Weising K, Fung RWM, Keeking DJ, Atkinson RG, Gardner R (1996) Characterization of microsatellites from *Actinidia chinensis*. Mol Breed 2:117–131