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Inheritance in turnip of variable-number tandem-repeat genetic markers revealed with synthetic repetitive DNA probes

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Abstract Oligomers (16–26 mers) composed of short, tandemly repeated DNA sequences (3–10 bases) were used individually with their complementary oligomer in separate polymerase chain reactions (PCRs) that extended the number of repeats to make 15 different PCR synthetic tandem-repeat (STR) probes. These PCR-STR probes were used to examine the inheritance of variable-number tandem-repeat (VNTR) genetic markers from two parent plants of turnip (*Brassica rapa* L.) to 20 offspring. Following *Hin*fI digestion and PCR-STR probing of Southern blots, interpretable variable parental and offspring band profiles were found with 9 of the 15 probes used. Each of these nine probes produced a unique set of fragments, and no cases of different probes revealing the same fragment were detected. Seventy-nine parental fragments were found and, of these, 65% (51) appeared to be heterozygous in one or both parents, with 52% (41) appearing to be heterozygous in one of the parents exclusively. That these fragments are transmitted as though heterozygous in the parents implies that they are derived from the nuclear complement of the genome. Chi-square analyses of the transmission of markers are, in general, consistent with Mendelian expectations, although three non-parental bands were found accounting for approximately 0.5% of these transmitted bands. For the fragments heterozygous in one of the parents exclusively, seven alleles exhibited complete linkage in three groups, 12 alleles were incompletely linked in six groups, and four allelic groups involving 11 alleles were identified. PCR-STR probes are relatively rapid to generate and apply (no cloning, clone screening, or sequencing steps are required), and have been shown to reveal VNTR genetic markers in a wide variety of plant species. These results add to the list of studies showing that VNTR genetic markers (and in this case, markers revealed by PCR-STR probes) are transmitted for the greater part in a Mendelian fashion.

Key words DNA fingerprinting · Linkage mapping
VNTR analysis · RFLP analysis · *Brassica rapa*

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Introduction

It has been demonstrated across a wide taxonomic range of organisms that a locus including a tandemly repeated sequence may exhibit moderate to high levels of intraspecific variation in the alleles available for that locus (Amos and Pemberton 1993). Such loci are thought to have alleles that differ in the number of repeats of the "core" sequence (Jeffreys et al. 1985 a, b), and thus have been termed variable-number tandem-repeat (VNTR) loci (Nakamura et al. 1987). Core sequences at VNTR loci may be long [e.g., greater than 100 basepairs (bp), as in macrosatellites (Jabs et al. 1989)], or shorter [e.g., 2–4 bp, as in simple sequence repeats or microsatellites (Ali et al. 1986; Lit and Luty 1989; Weber and May 1989); 5–100 bp for minisatellites (Jeffreys et al. 1985a, b)].

The first VNTR probes included repetitive sequences cloned from humans (Jeffreys et al. 1985 a, b; Nakamura et al. 1987), but minisatellite probes were subsequently found in other organisms such as the bacteriophage M13 repeat probe (Vassart et al. 1987) and the period (per) gene probe from *Drosophila* (Georges et al. 1987). Further, it was found that synthesized oligomer repeats, approximately 20 bases in length, used to probe intact gels (not Southern blots) revealed variation in a variety of organisms (e.g., Ali et al. 1986; Epplen 1988), while repetitive probes constructed by ligating short oligonucleotides composed of 14 randomly chosen nucleotides revealed numerous polymorphic loci in humans (Vergnaud 1989).

Because short oligomers, labeled and used as probes for Southern filter hybridizations, can often yield a high background or may hybridize differently relative to minute changes in temperature (Sambrook et al. 1989; personal

observation), a method was developed (Rogstad 1993) to use the polymerase chain reaction (PCR) to extend short, synthesized oligomers composed of tandem-repeats to obtain longer sequences (approximately 300–600 bp) composed of the same repeats. These longer PCR-synthetic tandem-repeat (PCR-STR) probes are then labeled and used in filter hybridizations similar to standard Southern-blot probing techniques. As demonstrated below, one filter may be examined with at least 15 different PCR-STR probes.

Several investigations have demonstrated that VNTR genetic markers are generally transmitted in a Mendelian fashion in a wide taxonomic range of species, including humans (Jeffreys et al. 1986), dogs (Jeffreys and Morton 1987), birds (Burke and Bruford 1987), butterflies (Saccheri and Bruford 1993), rice (Dallas 1988) and other organisms (e.g., see references in Amos and Pemberton 1993). Individual loci have been identified that exhibit extremely high rates of mutation, but such findings have not been common (Jeffreys et al. 1988.). In this study I have examined the transmission of VNTR markers detected with PCR-STR probes from a controlled cross of two parental individuals of the turnip (*Brassica rapa* L.; Brassicaceae) to 20 offspring.

Table 1 PCR-STR probe details

Core sequence	Reference	PCR conditions[a]	Hot wash conditions[b]
CAC	Epplen 1988	51;67	1.3 x;60
CACTCC	Random hexamer core	54;71	2 x;60
CCCAGT	Georges et al. 1987	45;65	2 x;55
CCTCCTGCCC	Jeffreys et al. 1985	50;70	2 x;60
GACA	Epplen 1988	47;65	1.8 x;60
GATA	Epplen 1988	47;65	2 x;60
GATGTGGG	Nakamura et al. 1987	56;69	1.8 x;60
GCAC	random tetramer core	55;71	2 x;60
GCTGGTGG	Jeffreys et al. 1985	46;65	1.5 x;60
M13	Vassart et al. 1987	53;70	2 x;60
TAA	Stop codon	49;67	2 x;60
TAAA	Brown et al. 1990	50;69	2 x;60
TAAG	Brown et al. 1990	50;69	2 x;60
TGAC	Random tetramer core	54;71	2 x;60
TTCCA	Fowler et al. 1987	50;69	1.5 x;60

[a] All PCR reactions included an initial treatment of 95°C for 1.5 min then 80°C for 1.5 min before adding polymerase and initiating the thermocycling. Denaturation was always at 95°C for 1 min 10 s. Numbers given are annealing temperature (1 min 10 s); and extension temperature (2 min), both in °C

[b] Details given are SSC concentration; and temperature (°C) of hot wash of Westneat et al. (1988)

Materials and methods

Sixteen plants of each of four different sources of turnip (two commercially available varieties, purple-top white globe and seven top, from both W. Atlee Burpee and Co. and Advance Seed Co.) were grown in the greenhouse. When sufficient leaves were available for non-destructive sampling, approximately 0.66 gm of clean leaf tissue was ground in liquid nitrogen and then extracted and gel-purified as described in Rogstad (1993). Genomic DNA from each plant was digested with three separate endonucleases (*Hae*III, *Hin*fI, *Taq*I; 5 µg DNA per digestion). Endonucleases were used according to manufacturer's specifications except that a five-fold excess of enzyme was employed. DNA from all plants digested with a particular enzyme were electrophoresed on the same 20×25 cm 1% agarose gel in 1×TBE at 2 V/cm until fragments less than 1000 bp were run off the gel. Southern transfers to nylon membranes (Micron Separations, Inc.) were conducted according to Sambrook et al. (1989), followed by UV crosslinking (Stratagene Stratalinker).

After probing the above filters with five PCR-STR probes (probing details are given below), two individuals that were among the plants most differentiated in their VNTR markers were selected for crossing. The pollen donor (staminate plant) was variety seven top and the pollen recipient (carpellate plant) was variety purple-top white globe (both plants were from W. Atlee Burpee and Co.). Turnips are biennials, so after 3 months of summer growth, these two plants were placed in a darkened cold room (4°C) and kept moderately moist for a further 3 months. The plants were then returned to separate greenhouses (in isolation from one another and any other turnip plant) with photoperiods of 14 h. For the carpellate plant, all flowers that were not to be used as pollen recipients were removed from the plant before they achieved sexual maturity. Those flowers that were to be used as pollen recipients were tagged and emasculated well before the anthers or stigmas matured. Over a period of 1 week, pollen from the staminate plant was collected daily into a microcentrifuge tube and was brushed onto the glistening stigmas of all emasculated, tagged flowers (these flowers were thus possibly pollinated on more than 1 day). Resultant seeds were harvested when the tagged fruit first began to show a change in color (from green to tan-brown) and became wrinkled. These seeds were stored at 4°C for 1 month and then planted. At the time of seed harvest, leaf tissue from both parents was ground in liquid nitrogen and stored at –70°C. When available, tissue from 20 randomly chosen offspring and from the two parents was extracted, digested, electrophoresed and transferred to membranes as described above to generate the filters used in the analyses discussed below. *Hin*fI was determined in the previous probing trials to reveal the clearest banding profiles and was used in these progeny analyses.

Membrane prehybridization, hybridization, and wash conditions followed Westneat et al. (1988), with the exceptions noted in Table 1. Still-moist filters were sealed in plastic wrap and autoradiography was performed at –70°C with intensifying screens for 1–12 days. Filters were stripped according to Sambrook et al. (1989).

PCR-STR probes were made and utilized according to Rogstad (1993). In brief, for a particular probe, an oligomer with 3–6 repeats of a template sequence, and its complement, were synthesized. Equimolar amounts of the template and complement are subjected to more or less standard, repeated DNA polymerase reactions. When strands are annealing, some of them anneal out of "register" in a fashion that permits 3′ end extension (with 5′ overhanging single-stranded DNA at each end), thus adding to the number of repeats of a strand. After repeated cycles, very long strands are generated. To facilitate only legitimate template-complement pairing, initial trials were conducted to determine the most stringent conditions under which extension will proceed. After repeated PCR extensions to create longer strands, the PCR-STR product is electrophoresed against a size marker and strands in the 300–600 bp size range are isolated. This size-selected PCR-STR probe is then labeled using asymmetric PCR with only one of the primers and ^{32}P-labeled nucleotides substituted for one or two of the four nucleotides. Complete details of the creation and use of PCR-STR probes are given in Rogstad (1993).

The PCR-STR probes used here were either designed after core sequences previously shown to reveal VNTR loci in other organisms or were chosen at random. Table 1 gives the core sequence of each probe, the reference from which the core sequence was taken, the PCR conditions used to generate the probe, and the conditions used for the hot wash of the Westneat et al. (1988) protocol.

Six types of parental fragments were recognized, including: (1) heterozygous in staminate, absent in carpellate; (2) heterozygous in carpellate, absent in staminate; (3) heterozygous in both parents; (4) homozygous in staminate, absent in carpellate; (5) homozygous in

carpellate, absent in staminate; and (6) present in both parents and homozygous in at least one of them. In types 1, 2, 4, and 5, only one parent will have the band. In types 1 and 2, transmission is expected to 50% of the offspring (10), while in types 4 and 5, transmission to 100% of the offspring (20) would be expected. When scoring autoradiographs, all cases where a band is present in only one of the parents, but occurs in all 20 offspring, are designated as types 4 or 5 since the expected probability of such transmission if the parent is heterozygous is 9.53×710^{-7} (Batschelet 1979; p. 435). Considering all cases where only one of the parents has a fragment and is designated heterozygous (types 1 or 2), the least number of offspring receiving a fragment was six and the greatest number was 15. In types 3 and 6, both parents would have the marker, but for the former, 75% of the offspring (15) are expected to receive it, while for the latter, transmission to 100% is expected. In addition, a seventh type of fragment, non-parental (bands appearing in offspring that are not present in the parents), was found. In scoring the transmission of markers, at least three autoradiographs of varying intensity for each probing were usually available. Chi-square analysis of transmission of markers heterozygous in only one or in both parents was performed according to Weir (1990; p. 24). The SYSTAT (Wilkinson 1984) and LINKAGE (Ott 1993) programs were used as described below to search for linked alleles.

Results

Examples of fragment profiles revealed with different PCR-STR probes are shown in Figs. 1–3, and ranged from very simple (Fig. 1) to quite complex (Figs. 2–3). Each probe that revealed fragments produced a set of unique fragments when compared to the other probes. No cases were detected where different probes revealed the same fragment (or fragments). Scoring of Figs. 1 and 2 are given in Table 2 to demonstrate aspects of data collection and classification. The figures also demonstrate fragments that could not be scored. For example, in Fig. 2 there is a light fragment that comigrates with a darker fragment at the fifth fragment position. In such cases, only the darker band could be scored since its presence might obscure the presence of the lighter band. Occasionally, fragments would be clearly present in some individuals but were difficult to interpret for others (see fragment between scorable frag-

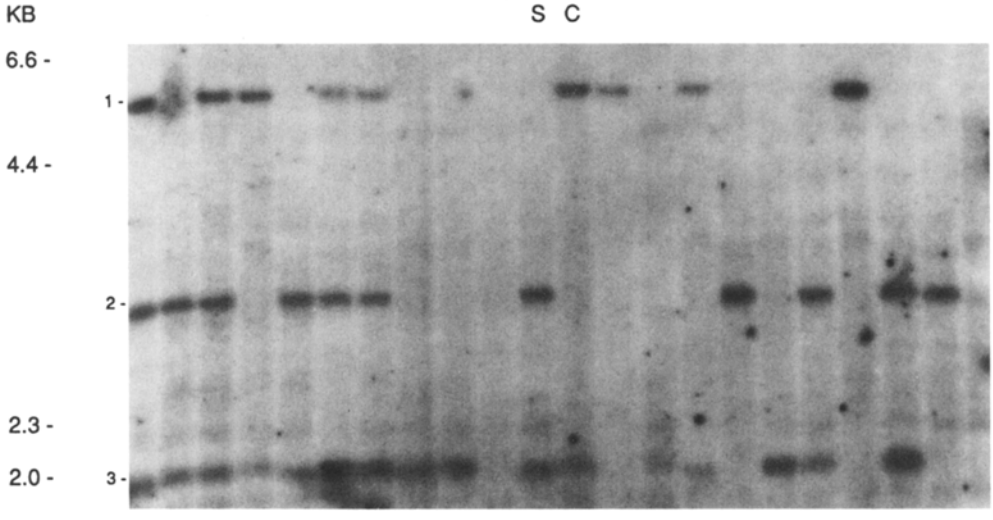

Fig. 1 *Hin*fI fragments revealed with a PCR-STR probe composed of repeats of TTCCA in the 20 offspring from a cross of the staminate (*S*) and carpellate (*C*) plants of *Brassica rapa*. Only scorable parental fragments are marked. *KB* kilobase pairs

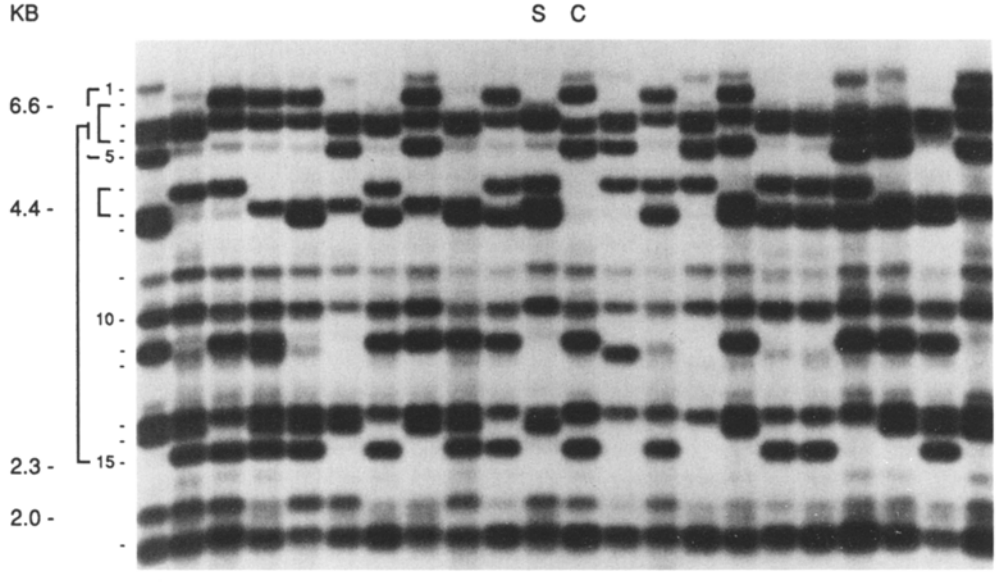

Fig. 2 *Hin*fI fragments revealed with a PCR-STR probe composed of repeats of GATA in the 20 offspring from a cross of the staminate (*S*) and carpellate (*C*) plants of *B. rapa*. Only scorable fragments are marked. *Dashed lines* connect completely linked fragments; *solid lines* connect allelic fragments. *KB* kilobase pairs

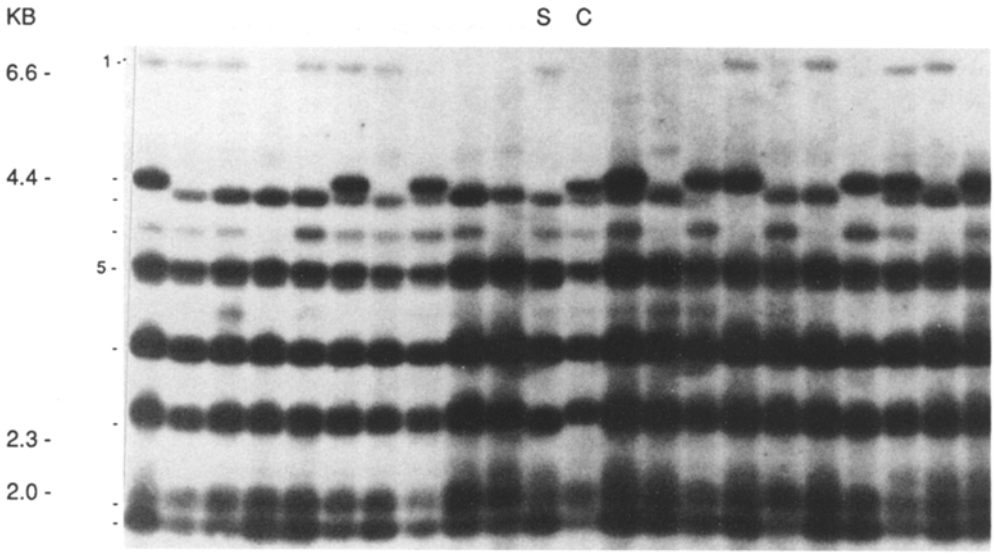

Fig. 3 *Hin*fI fragments revealed with a PCR-STR probe composed of repeats of CCCAGT in the 20 offspring from a cross of the staminate (*S*) and carpellate (*C*) plants of *B. rapa*. Only scorable parental fragments are marked. *KB* kilobase pairs

Table 2 Scoring of Figs. 1–2. 0 fragment not present; 1 fragment present. Type of transmission is defined in the text

Markers	Offspring 1–10	Parent		Offspring 11–20	Markers transmitted	Transmission type
		Staminate	Carpellate			
Fig. 1						
1	1011011000	0	1	1010001000	8	2
2	1110111000	1	0	0001010110	10	1
3	1111111110	1	1	0000110100	12	3
Fig. 2						
1	1000010100	0	1	1011001101	9	2
2	0011100101	0	1	0101000001	8	2
3	1111111111	1	0	1111111111	20	4
4	1100011010	0	1	1010111110	12	2
5	1000010100	0	1	1011001101	9	2
6	0110001001	1	0	1110111000	10	1
7	1001110110	1	0	0001000111	10	1
8	1000101011	1	0	0101111110	12	1
9	1111111111	1	1	1111111111	20	6
10	1111111111	1	1	1111111111	20	6
11	1011001111	0	1	0001001110	11	2
12	0001000000	0	0	1000000000	2	7
13	1111111111	1	1	1111111111	20	6
14	1101110110	1	0	0001001110	11	1
15	0111101011	0	1	0100110010	11	2
16	1111111111	1	1	1111111111	20	6

ments 15 and 16), and such bands have been excluded from the analysis.

Of the 15 probes employed under the given conditions (Table 1), three designed on variations of the stop codon (core sequences TAA, TAAA, and TAAG; Brown et al. 1990) did not significantly cross-hybridize with any turnip fragments; two other probes (M13 and core TGAC) hybridized so abundantly that fragments could not be interpreted; and the remaining nine produced decipherable fragments. For these nine probes, the parental bands were classified for each probe into the different types mentioned above, the results being given in Table 3 which also includes a chi-square analysis of these data. The nine probes differed in the degree to which informative bands were revealed, the TTCCA repeat probe producing the fewest interpretable fragments (3), while the GCTGGTGG repeat probe produced the most (14).

Table 3 shows that, across all probes, 79 parental fragments were scorable. Of these, 9% (eight fragments) were present and homozygous in only one or the other parent, 25% (20 fragments) were apparently homozygous in both parents (or homozygous in one and heterozygous in the other), 65% (51 fragments) were heterozygous in one or both parents, and 52% (41 fragments) were present and heterozygous in one parent alone. The parents do not differ from one another significantly [0.05 significance level; z test of Noether (1976), p. 91] in the number of heterozygous fragments occurring in only one of the parents

Table 3 Chi-square analyses of turnip cross-parental VNTR marker transmission to 20 offspring for different PCR-STR probes. For the genotypes, a 0/0, 0/1, or 1/1 indicates that a parent was homozygous null, heterozygous, or homozygous, respectively, for a given marker (autoradiograph band). For transmission, the column marked 1 indicates the offspring receiving a parental band, and the column marked 0 indicates offspring lacking the parental band. Under the chi-square test for two categories (giving 1 *df*), there is only a 5% chance that chi-square will exceed 3.84 (indicated by an asterisk) if the hypothesis of Mendelian transmission is true (Weir 1990, p. 24). See text for further explanation

Core sequence	Parental genotypes		Transmission Observed/Expected[a]		Chi-square
	Staminate	Carpellate	1	0	
CAC	0/0	0/1	8/10	12/10	0.800
	0/0	0/1	14/10	6/10	3.200
	0/1	0/0	12/10	8/10	0.800
	0/1	0/0	15/10	5/10	5.000*
	0/1	0/1	9/15	11/5	9.600*
	0/1	0/1	16/15	4/5	0.267
	1/1	1/1	20/20	0/0 (4)	–[b]
CACTCC	0/0	0/1	9/10	11/10	0.200
	0/1	0/0	8/10	12/10	0.800
	0/1	0/0	10/10	10/10	0.000
	0/0	1/1	20/20	0/0 (2)	0.000
	1/1	1/1	20/20	0/0 (2)	–[b]
CCCAGT	0/0	0/1	9/10	11/10	0.200
	0/1	0/0	10/10	10/10	0.000
	0/1	0/1	14/15	6/5	0.267
	0/1	0/1	16/15	4/5	0.267
	1/1	1/1	20/20	0/0 (5)	–[b]
CCTCCTGCCC	0/0	0/1	11/10	9/10	0.200
	0/1	0/0	9/10	11/10	0.200
	0/0	1/1	20/20	0/0 (2)	0.000
GACA	0/1	0/0	9/10	11/10	0.200
	0/1	0/0	11/10	9/10	0.200
	0/1	0/1	17/15	3/5	1.067
GATA	0/0	0/1	8/10	12/10	0.800
	0/0	0/1	9/10	11/10 (2)	0.200
	0/0	0/1	11/10	9/10 (2)	0.200
	0/0	0/1	12/10	8/10	0.800
	0/1	0/0	10/10	10/10 (2)	0.000
	0/1	0/0	11/10	9/10	0.200
	0/1	0/0	12/10	8/10	0.800
	1/1	0/0	20/20	0/0	0.000
	1/1	1/1	20/20	0/0 (4)	–[b]
GATGTGGG	0/0	0/1	9/10	11/10	0.200
	0/0	0/1	12/10	8/10	0.800
	0/0	0/1	13/10	7/10	1.800
	0/0	0/1	15/10	5/10	5.000*
	0/1	0/0	12/10	8/10	0.800
	0/1	0/1	19/15	1/5	4.267*
	1/1	1/1	20/20	0/0 (3)	–[b]
GCAC	0/0	0/1	6/10	14/10	3.200
	0/0	0/1	15/10	5/10	5.000*
	0/1	0/0	9/10	11/10	0.200
	1/1	0/0	20/20	0/0 (2)	0.000
GCTGGTGG	0/0	0/1	9/10	11/10	0.200
	0/0	0/1	11/10	9/10 (3)	0.200
	0/0	0/1	12/10	8/10 (2)	0.800
	0/1	0/0	8/10	12/10	0.800
	0/1	0/0	15/10	5/10	5.000*
	0/1	0/1	14/15	6/5	0.267
	0/1	0/1	16/15	4/5 (2)	0.267
	1/1	0/0	20/20	0/0	0.000
	1/1	1/1	20/20	0/0 (2)	–[b]
TTCCA	0/0	0/1	8/10	12/10	0.800
	0/1	0/0	10/10	10/10	0.000
	0/1	0/1	12/15	8/5	2.400

[a] The number of loci for a probe with transmission to the indicated number of offspring is given in parentheses if it is greater than one

[b] The class scored as both parents homozygous for a marker might also be scored homozygous in one parent and heterozygous in the other. Since this status is not known, and because a mutation of such an allele might go undetected in these offspring analyses, alleles of this class are not included in the chi-square analysis

(z=–1.093), or if all heterozygous fragments are considered (n=61; z=–0.897).

Table 3 shows that fragments heterozygous in only the staminate plant were transmitted on average to 53.2% of the offspring (mean number of offspring over 17 loci=10.65; SD=2.06), while transmission of fragments heterozygous in only the carpellate plant averaged 52.9% of the offspring (mean over 24 loci=10.58; SD=2.28). For both types of these markers combined, transmission was to 53.1% of the offspring (mean over 41 loci=10.61; SD=2.17). For fragments that were shared by and heterozygous for both parents, detectable transmission is expected to 75% (15) of the offspring. Out of the ten loci where this type of fragment was detected, an average of 14.6 offspring (73%; SD=2.91) received at least one copy of the fragment. Chi-square analyses (Table 3) are, in general, consistent with Mendelian transmission of these markers. There were six exceptions, but given the number of segregations that could be tested (59), three significant deviations from the expected should occur on average if the null hypothesis were true.

The program LINKAGE (Ott 1993) was used to assist in determining whether different fragments present and heterozygous in one of the parents exclusively exhibited complete linkage (two different fragments passed to exactly the same offspring) or incomplete linkage (two different parental fragments that differed in transmission by more than two offspring could not be declared to be significantly linked since lod scores in such cases are ≥3). An example of two parental fragments exhibiting complete linkage is shown in Fig. 2, where the first and fifth bands are completely linked. For the fragments present and heterozygous in one of the parents exclusively, seven exhibited complete linkage in three groups (two staminate; one carpellate) and, in some cases, two different probes revealed completely linked fragments (e.g., the second band of Fig. 1 is completely linked to the first band of Fig. 3; bands 1 and 5 of Fig. 2 are completely linked to band 2 of Fig. 3). In all cases of completely linked fragments found with two different probes, the different fragments are clearly of different sizes (see examples just mentioned) demonstrating that the two probes were not just revealing the same fragment. Further, 12 fragments exhibited incomplete linkage in six groups.

Allelic bands (i.e., bands that always segregate A- and -B) were also investigated for fragments heterozygous in only one parent. For example, allelic bands are connected by solid lines in Fig. 2. Allelic bands were also found across two probes. In all, four cases of allelism (two staminate; two carpellate) involving 11 loci were revealed. As expected, all fragments that were linked or were allelic were from the same parent.

The following anomalies were also detected. Two nonparental bands involving three individuals were found (band 12 of Fig. 2 in two offspring; a band between fragments 1 and 2 in the 12th offspring from the left in Fig. 3). Given that, including heterozygous and homozygous fragments restricted to one or the other parents, 595 fragments (261 staminate; 334 carpellate) were shown above to be transmitted in agreement with Mendelian expectations, the three non-Mendelian fragments account for only 0.50% of that data set.

Discussion

New methodologies for the analysis of DNA variation are bringing about a revolution in the study of linkage mapping and population genetics. Ideally, what is required for such analyses is a set of genetic markers for loci that are (1) highly variable [e.g., exhibit high heterozygosity (Nakamura et al. 1987)]; (2) widely distributed across the nuclear genome; (3) transmitted in a Mendelian fashion; and (4) easily obtained for any given species. This study demonstrates that PCR-STR probes are a new tool that offers these characteristics.

First, a high percentage of the loci detected were heterozygous, and employing 15 probes, 51 heterozygous loci were revealed. The cultivars used probably have a history of at least some inbreeding, and thus levels of heterozygosity may be even higher in some natural populations.

Second, including loci at which both parents were heterozygous for the same fragment, but subtracting the repetitive cases of fragments that are linked or allelic, 39 different and independently assorting heterozygous loci were detected. The relatively low number of allelic fragments implies that alternative alleles for most of the fragments revealed are shorter and located in the unresolvable portions of the autoradiographs, a pattern similar to that for VNTR loci in humans (Jeffreys et al. 1986). For the turnip, 2n=20 (Crane and Lawrence 1956), and while some of these 39 loci may be clustered to some degree, their apparently independent assortment suggests that they may be widely distributed over the genome (see arguments in Jeffreys et al. 1986, p. 18). The transmission of alleles at these loci implies heterozygosity, thus supporting the hypothesis that they are nuclear as opposed to mitochondrial or chloroplast DNA. Usually in the latter two cases, all offspring would have a DNA fragment from only one of the parents, similar to the case where one of the parents is homozygous for a fragment lacking in the other parent (note, however, that in four such cases, transmission is from the staminate plant). Further, these autoradiographs are produced under conditions used for single-copy probing (e.g., 5 μg DNA electrophoresed per individual), and extensive repetitive DNA is generally lacking from organellar DNA (Avise 1994).

Third, the fragments are for the greater part transmitted in accordance with Mendelian expectations. The detectable occurrence of non-Mendelian bands is probably related to the fact that these genetic markers are among the most variable yet known.

Fourth, PCR-STR probes can be applied successfully across a wide taxonomic range of species. Previously these probes have been shown to reveal variation in members of other plant families (e.g., Annonaceae, Aspleniaceae, Asteraceae, Equisetaceae, Hippocastanaceae, Lauraceae, and

Moraceae; Rogstad 1993 and personal observation). While it is unpredictable which probes will reveal variation in which species, the array of potential PCR-STR probes is virtually limitless, and probes revealing variation are usually found for most species explored thus far after a few probings. Experience has shown that some probes are less widely useful in revealing variation than others. For example, the probes based on variations of the stop codon (TAA, TAAA, and TAAG) were not only of little utility with turnip, but have revealed little or no fragments with other taxa (including species in Annonaceae, Asteraceae, Hippocastanaceae, Lauraceae, and Rosaceae). Similar poor results have been obtained with probes designed from repeated cores that will hybridize to themselves (e.g., GATC, GTAC, AGCT, etc.). Systematic studies are needed to determine if some probes are more universally applicable or non-informative than others.

In conclusion, although microsatellite analysis (Litt and Luty 1989; Weber and May 1989) may be the currently preferred method for finding and utilizing genetic intraspecific variation, this approach is only readily adaptable to species for which a great deal of sequence information has already been accumulated, otherwise involving extensive cloning, sequencing, DNA amplification, etc., steps. VNTR genetic markers have proven of utility in forensics, pedigree analysis, linkage mapping, cell and organ typing, in situ chromosomal hybridization, sex determination, and various aspects of population genetics (e.g., see Amos and Pemberton 1993; Avise 1994). Further, expansion of trinucleotide repeats has recently been demonstrated to be involved in several independent types of human genetic disease (Davies 1993), and the PCR-STR method provides a source of probes to screen clones or whole genomic blots for the occurrence of such repeats. It would not be surprising if expansion of such repeats was involved in related genetic phenomena in other organisms. In cases where no sequence information is at hand, PCR-STR probes provide another tool by which the molecular geneticist can search for genetic variation in a relatively rapid fashion.

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References

Ali S, Muller CR, Epplen JT (1986) DNA fingerprinting by oligonucleotide probes specific for simple repeats. Hum Genet 74:239–243

Amos B, Pemberton J (1993) A bibliography of DNA fingerprinting studies. Fingerprint News 5:2–64

Avise JC (1994) Molecular markers, natural history, and evolution. Chapman and Hall, New York

Batschelet E (1979) Introduction to mathematics for life scientists, 3rd edn. Springer-Verlag, New York

Brown CM, Stockwell PA, Trotman CNA, Tate WP (1990) Sequence analysis suggests that tetra-nucleotides signal the termination of protein synthesis in eukaryotes. Nucleic Acids Res 18:6339–6347

Burke T, Bruford MW (1987) DNA fingerprinting in birds. Nature 327:149–152

Crane MB, Lawrence WJC (1956) The genetics of garden plants. MacMillan and Co., New York

Dallas JF (1988) Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite DNA probe. Proc Natl Acad Sci USA 85:6831–6835

Davies K (1993) Triplet repeats on the rise. Nature 364:88

Epplen JT (1988) On simple repeated GATA-GACA sequences in animal genomes: a critical reappraisal. J Hered 79:409–417

Fowler C, Drinkwater R, Burgoyne L, Skinner J (1987) Hypervariable lengths of human DNA associated with a human satellite-III sequence found in the 3.4-kb Y-specific fragment. Nucleic Acids Res 15:3929

Georges M, Cochaux P, Lequarre A-S, Young MW, Vassart G (1987) DNA fingerprinting in man using a mouse probe related to part of the *Drosophila* 'per' gene. Nucleic Acids Res 15:7193

Jabs EW, Goble CA, Cutting GR (1989) Macromolecular organization of human centromeric regions reveals high-frequency, polymorphic macro-DNA repeats. Proc Natl Acad Sci USA 86: 202–206

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18:1–15

Jeffreys AJ, Wilson V, Thein SW (1985 a) Hypervariable 'minisatellite' regions in human DNA. Nature 314:67–73

Jeffreys AJ, Wilson V, Thein SW (1985 b) Individual-specific 'fingerprints' of human DNA. Nature 316:76–79

Jeffreys AJ, Wilson V, Thein SL, Weatherall DJ, Ponder BAJ (1986) DNA "Fingerprints" and segregation analysis of multiple markers in human pedigrees. Am J Hum Genet 39:11–24

Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 332:278–281

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E, White R (1987) Variable number of tandem-repeat (VNTR) markers for human gene mapping. Science 235:1616–1622

Noether GE (1976) Introduction to statistics. Houghton Mifflin Co., Boston, Massachusetts

Ott J (1993) LINKAGE, version 5.2. Available from J. Ott, Columbia University, New York

Rogstad SH (1993) Surveying plant genomes for variable number of tandem-repeat loci. Methods Enzymol 224:278–294

Saccheri IJ, Bruford MW (1993) DNA fingerprinting in a butterfly, *Bicyclus anynana* (Satyridae). J Hered 84:195–200

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. Cold Spring Harbor Laboratory , Cold Spring Harbor, New York

Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AS, Christophe D (1987) A sequence of M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235:683–684

Vergnaud G (1989) Polymers of random short oligonucleotides detect polymorphic loci in the human genome. Nucleic Acids Res 17:7623–7630

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weir BS (1990) Genetic data analysis. Sinauer Associates, Inc., Sunderland, Massachusetts

Westneat DF, Noon WA, Reeve HK, Aquadro CF (1988) Improved hybridization conditions for DNA 'fingerprints' probed with M13. Nucleic Acids Res 16:4161

Wilkinson L (1984) SYSTAT manual. SYSTAT Inc., Evanston, Illinois