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Inheritance and reliability of random amplified polymorphic DNA-markers in two consecutive generations of common carp (*Cyprinus carpio* L.)

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Abstract

Random amplified polymorphic DNA (RAPD) markers have been used in a variety of genetic studies in fisheries and aquaculture. Most population studies are performed without preliminary data demonstrating the Mendelian inheritance and reproducibility of RAPD markers. In this study, the inheritance and reproducibility of RAPD markers was examined in two consecutive generations of common carp, *Cyprinus carpio* L. Variability and segregation of RAPD markers were investigated in one F_1 progeny and three F_2 progenies. Seventy-four RAPD markers were generated by five primers using DNA extracted from the initial ornamental (koi) common carp female and wild-type colour common carp male. Fifty-five of these RAPD markers were transmitted to the F_1 progeny and the inheritance patterns were analysed. Twenty RAPD markers were fully reproducible and demonstrated dominant simple Mendelian inheritance patterns in two consecutive generations. Twenty-four RAPD markers were not reproducible in all progenies. Thirteen markers displayed inheritance ratios in the progenies that did not fit simple Mendelian inheritance patterns. Non-reproducibility of RAPD markers and distorted ratios may be caused by the absence of amplification, poor amplification or by the appearance of artefact bands. Random amplified polymorphic DNA markers with poor reproducibility and non-Mendelian inheritance can lead to misinterpretations of data in population studies, resulting in errors in the estimation of genetic diversity within and between individual populations. Therefore, it is recommended to first identify the set of reproducible RAPD markers that demonstrate Mendelian inheritance before application of the RAPD technique in population studies.

Keywords: DNA markers, RAPD, inheritance, common carp

Introduction

The random amplified polymorphic DNA (RAPD) technique is an inexpensive procedure for generating polymorphic DNA markers for genetic studies. No prior genome sequence information is required to produce RAPD markers (Welsh & McClelland 1990; Williams, Kubelik, Livak, Rafalski & Tingey 1990). The ease of use of the RAPD technique has led to numerous genetic studies with plant, animal and fish species (Penner 1996; Bardakci 2001; Ali, Huang, Qin & Wang 2004; Salem, Ali, Huang & Qin 2005).

The RAPD marker technique has been widely used in aquaculture and fisheries in the evaluation of the genetic variation and diversity of cultivated stocks and wild populations (Yoon & Kim 2001; Hatanaka & Galetti Jr. 2003; Hassanien, Elnady, Obeida & Itriby 2004; Das, Prasad, Meher, Barat & Jana 2005; Ramella, Kroth, Meurer, Nuñer, Filho & Arisi 2006). Random amplified polymorphic DNA markers have been used to examine the genetic diversity in common carp *Cyrpinus carpio* L. (Dong & Zhou 1998; Bártfai, Egedi, Yue, Kovács, Urbányi, Tamás, Horváth, & Orbán 2003; Wang & Li 2004; Ludanny, Christanfova,

Vasileyev, Prizenko, Bogeruk, Ryskov, & Semenova 2006; Basavaraju, Prasad, Rani, Kumar, Naika, Jahageerdar, Srivastava, Penman & Mair 2007). The RAPD technique has also been used to construct the first genetic linkage map for common carp and has been used to identify a locus associated with cold tolerance (Sun & Liang 2004).

Even though the RAPD technique is widely used in aquaculture and fisheries, there have been few studies examining the inheritance of RAPD markers in fish, especially in consecutive generations, and there have not been any inheritance studies in common carp. Most population studies performed with RAPD markers are conducted without preliminary data demonstrating marker inheritance and reproducibility. However, commonly observed problems with RAPD marker analysis are poor reproducibility, deviation in the observed segregations from expected ratios and the appearance of non-parental bands (Stott, Ihssen & White 1997; Appleyard & Mather 2000; Bardakci 2001; Ali *et al.* 2004; Liu & Cordes 2004). These problems can be reduced or avoided by using selected markers resulting from prior inheritance and reproducibility studies. The objective of this study was to investigate the inheritance and reproducibility of RAPD markers in two consecutive generations in the common carp.

Materials and methods

Scheme of crosses and fish rearing

The inheritance of RAPD markers was examined in two consecutive generations. The first-generation progeny was obtained by crossing of an ornamental (koi) common carp female from koi broodstock at the Aquaculture Research Center at Kentucky State University, with the wild-type colour common carp male collected at a local lake in Frankfort, Kentucky. Two genetically distant fish were chosen for the initial cross in order to increase the variability of RAPD markers in the F_1 progeny. The first-generation progeny was maintained in ponds until the fish reached sexual maturity at 2 years of age. Three randomly chosen sets of F_1 females and males were used in single-paired crosses to produce three progenies of F_2. The variability and segregation of RAPD markers were investigated in the initial parents, one F_1 progeny and three F_2 progenies.

To induce final oocyte maturation or spermiation, fish parents were injected with Carp Pituitary Extract (Sigma Chemical, St Louis, MO, USA) at 3 mg kg^{-1}. Eggs were artificially inseminated in 1.5 L glass trays, which were placed for embryo incubation in a tank system with 50–75% daily water exchange. After transition to active feeding, larvae from the F_1 progeny were fed with *Artemia* nauplii for 7 days and were then stocked in a 0.04 ha pond for further rearing until fish reached sexual maturity. Larvae from F_2 progenies were fed with *Artemia* nauplii, zooplankton and artificial diets for 6 weeks.

Fin clips were taken for DNA analysis from the initial parents and from 30–36 fish from the first-generation progeny and were preserved in 95% ethanol. Six-week-old fry from the second generation were also taken and preserved in 95% ethanol until DNA extraction.

DNA extraction

DNA samples were extracted using the mouse tail extraction protocol of the Promega Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) with one minor modification: the initial incubation period (65 °C instead of 55 °C). The DNA concentration and 260/280 nm absorbance ratios were determined using the GeneQuant *pro* RNA/DNA Calculator (Biochrom, Cambridge, UK). DNA stocks displayed 260/280 nm absorbance ratios between 1.7 and 1.9. The samples were diluted to a DNA concentration of 1 ng μL^{-1}, and stored at 4 °C for use in polymerase chain reactions (PCR).

RAPD primers

Thirty-eight RAPD primers from the University of British Columbia (UBC) RAPD Primer Set #2 (University of British Columbia, Vancouver, Canada) were screened to amplify DNA samples of the initial female (koi) and male (wild-type colour) common carp. Eighteen primers were selected to test for the reproducibility of band phenotypes in the initial parents. Five primers (UBC109, UBC112, UBC120, UBC152 and UBC154) were selected for further investigation of inheritance of RAPD markers. Specific parental band profiles were generated by these five primers in at least three replicate PCR.

PCR reactions

All PCR reactions contained 4 ng of template DNA in a total reaction volume of 20 μL containing a final

concentration of 1 × PCR buffer, 0.25 mM dNTP's, 3.1 mM $MgCl_2$, 0.195 μM primer and 0.1 U *Taq* polymerase (Promega). Amplifications were performed using two Gene Amp® System 9700 thermal cyclers (Applied Biosystems, Foster City, CA, USA). The temperature profile for all reactions consisted of an initial denaturing period of 94 °C for 5 min, followed by 45 cycles of 1 min at 95 °C, 1 min 30 s at 36 °C, 30 s at 54 °C and 2 min at 72 °C, with a final extension of 15 min at 72 °C.

Amplified products were separated by electrophoresis on a 300 mL 1.5% agarose gel in 1/2 × TBE buffer at 120 V applied for approximately 4 h. Each sample that was loaded onto the agarose gels for separation consisted of 16 μL of amplified DNA and 4 μL of loading dye.

Gel electrophoresis

All F_1 replicate gels for each of the five selected primers contained samples of amplified genomic DNA from the initial female (koi), the initial male (wild-type colour), 32 F_1 fish and also the Hyperladder I DNA ladder (Bioline USA, Randolph, MA, USA). All F_2 replicate gels for each of the five selected primers contained samples of amplified DNA from the initial female (koi), the initial male (wild-type colour), the F_1 female and male parents, 30 fish for each of the three F_2 progenies and also the Hyperladder I DNA ladder.

The gels were stained with ethidium bromide and visualized on an ultra-violet light transilluminator. A digital photo of each gel was obtained using a Kodak DC120 digital camera (Kodak, Rochester, NY, USA). At least two replicate PCR amplifications and gels were completed for each of the selected primers to investigate the inheritance of markers in the first- and second-generation progenies. Two Gene Amp® System 9700 thermal cyclers were used for DNA amplification, with each unit being used for approximately 50% of replicate PCR amplifications.

Analysis of segregation

Random amplified polymorphic DNA markers were scored using the photo analysis software program KODAK 1D v3.5.0 (Eastman Kodak). Marker loci were described by the manufacturer's primer code, followed by a subscript, which represents the fragment size in base pairs.

The RAPD technique is a dominant DNA marker system. Therefore, if a band is present, the phenotype can be homozygous (+/+) or heterozygous (+/−); when the band is absent the phenotype is homozygous for recessive allele (−/−). When one parent displays an RAPD band and it is absent in the other parent, the genotypic crosses were +/+ × −/− or +/− × −/−. If the polymorphism for this locus resulted in a segregation 1:0 in the offspring, the parental genotype was homozygous for the dominant allele (+/+) and all the offspring were heterozygous (+/−). If the offspring resulted in a 1:1 segregation, it indicated that the parent, which had the band, was heterozygous (+/−) and that the other parent was homozygous (−/−). When both parents expressed the band present phenotype, each of them could either be homozygous for the dominant allele (+/+) or heterozygous (+/−). Heterozygosity in both parents would result in a 3:1 ratio in progeny. Two other possible crosses (+/+ × +/+ or +/+ × +/−) result in a 1:0 segregation. The observed segregation in progenies was compared with theoretical or expected ratios using χ^2 analysis for goodness of fit with Yates' correction for continuity (Zar 1999), and differences were considered to be significant at $P < 0.05$.

Results

Seventy-four RAPD bands were generated by five primers in the DNA profiles of the initial koi female and common carp male. Twenty-four markers were specific for the female, 17 were specific for the male and 33 markers were common for both fish parents (Table 1). Fifty-five RAPD markers were transmitted from initial parents to F_1 fish and their inheritance was analysed. In general, these RAPD markers could be divided into three major categories with regard to their inheritance and reproducibility. The RAPD markers of the first category fit theoretical simple Mendelian inheritance patterns for dominant markers and were reproducible in all replicates of the F_1 and F_2 progenies. The RAPD markers of the second category fit theoretical simple Mendelian inheritance patterns, but were not reproducible in all progenies. Finally, the RAPD markers of the third category did not fit simple Mendelian inheritance patterns in at least one progeny.

Segregation patterns for twenty RAPD markers, which showed Mendelian inheritance and were reproducible in all the progenies tested, are shown in Table 2. These RAPD markers were reproducible irrespective of whether the same or different thermal cyclers were used for replicate PCR amplifications. In

seven crosses, both the initial parents had corresponding bands. Four RAPD markers (UBC120-0607, UBC152-1126, UBC152-0869 and UBC152-0478) were monomorphic for band presence in F_1 progeny and all three F_2 progenies. This mode of inheritance pattern indicated that at least one of the initial parents was homozygous for the dominant allele (+/+) while the second parent could be homozygous for the dominant allele or heterozygous (+/−). In the case of heterozygosity in one of the initial parents, the ratios 3:1 could appear in F_2, if two heterozygous F_1 parents were crossed. For markers UBC109-1082 and UBC152-0916, all F_1 fish had a corresponding band while in F_2 ratios 3:1 or 1:0 were observed (Table 2). This mode of inheritance shows that the initial parents in these crosses had +/+ and +/− genotypes. The F_1 progenies consisted of fish +/+ and +/− with ratio 1:1; in F_2 progenies, the ratios 1:0 (all fish had band) or 3:1 appeared depending on the genotypes of F_1 parents randomly chosen for crosses. For marker UBC109-1025, the ratio 3:1 was observed in both F_1 progeny and all F_2 progenies. This indicated

Table 1 Characteristics of random amplified polymorphic DNA markers generated by five UBC primers in two consecutive generations of common carp

Primer	Markers specific for male parent	Markers specific for female parent	Common markers for both parents	Total	Transmitted markers	Fit Mendelian inheritance and fully reproducible	Fit Mendelian inheritance and not fully reproducible	Did not fit Mendelian inheritance
UBC109	3	4	3	10	10	5	2	3
UBC112	5	1	6	12	8	3	4	1
UBC120	6	4	11	21	11	2	7	3*
UBC152	2	2	7	11	10	5	3	3*
UBC154	8	6	6	20	16	5	8	3
	24	17	33	74	55	20	24	13*

*Including non-parental markers in F_1 (one for UBC120 and one for UBC152).

Table 2 Observed (OBS) segregations (band present:band absent) of reproducible random amplified polymorphic DNA markers which were not significantly different ($P > 0.05$) from expected (EXP) Mendelian ratios (1:1, 3:1, 1:0 or 0:1) in one F_1 and three F_2 progenies of common carp

	P	F_1		$F_1 \times F_1/1$	F_2-1		$F_1 \times F_1/2$	F_2-2		$F_1 \times F_1/3$	F_2-3	
Marker	♀/♂	OBS	EXP	♀/♂	OBS	EXP	♀/♂	OBS	EXP	♀/♂	OBS	EXP
109-1740	−/+	15:17	1:1	+/+	22:9	3:1	+/−	16:18	1:1	−/−	0:30	0:1
109-1082	+/+	32:0	1:0	+/+	22:9	3:1	+/+	28:6	3:1	+/+	30:0	1:0
109-1025	+/+	29:3	3:1	+/+	20:9	3:1	+/+	30:4	3:1	+/+	24:6	3:1
109-0803	+/−	15:17	1:1	−/+	17:14	1:1	+/−	19:15	1:1	+/+	25:5	3:1
109-0754	−/+	32:0	1:0	+/+	20:11	3:1	+/+	28:6	3:1	+/+	26:4	3:1
112-1962	+/−	15:18	1:1	−/−	0:34	0:1	+/−	18:15	1:1	−/−	0:30	0:1
112-0655	+/−	33:0	1:0	+/+	25:9	3:1	+/+	26:7	3:1	+/+	22:8	3:1
112-0398	+/−	33:0	1:0	+/+	29:5	3:1	+/+	28:5	3:1	+/+	25:5	3:1
120-0763	+/−	32:0	1:0	+/+	26:8	3:1	+/+	25:8	3:1	+/+	22:8	3:1
120-0607	+/+	32:0	1:0	+/+	34:0	1:0	+/+	33:0	1:0	+/+	30:0	1:0
152-1126	+/+	36:0	1:0	+/+	30:0	1:0	+/+	34:0	1:0	+/+	30:0	1:0
152-0916	+/+	36:0	1:0	+/+	30:0	1:0	+/+	25:9	3:1	+/+	30:0	1:0
152-0869	+/+	36:0	1:0	+/+	30:0	1:0	+/+	34:0	1:0	+/+	30:0	1:0
152-0776	−/+	36:0	1:0	+/+	23:7	3:1	+/+	25:9	3:1	+/+	25:5	3:1
152-0478	+/+	36:0	1:0	+/+	30:0	1:0	+/+	34:0	1:0	+/+	30:0	1:0
154-0793	+/−	15:15	1:1	+/−	14:17	1:1	+/−	14:20	1:1	+/−	17:15	1:1
154-0764	+/−	15:15	1:1	−/+	12:19	1:1	+/−	14:20	1:1	−/+	19:14	1:1
154-0749	−/+	31:0	1:0	+/+	21:10	3:1	+/+	29:5	3:1	+/+	28:5	3:1
154-0492	+/−	17:14	1:1	+/−	14:17	1:1	−/−	0:34	0:1	−/−	0:31	0:1
154-0449	+/−	30:0	1:0	+/+	27.4	3:1	+/+	23:11	3:1	+/+	26:7	3:1

Table 3 Observed (OBS) segregation of random amplified polymorphic DNA markers which fit expected (EXP) segregations in some progenies but demonstrated non-reproducible (NR) readings in other progenies

	P	F_1		$F_1 \times F_1/1$	F_2-1		$F_1 \times F_1/2$	F_2-2		$F_1 \times F_1/3$	F_2-3	
Marker	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**
109-0887	−/+	18:14	1:1	+/+	23:8	3:1		NR		+/+	NR	
109-0964	+/+	29:3	3:1	+/+	24:7	3:1	+/+	30:4	3:1	+/+	NR	
112-0941	+/+	28:4	3:1	+/+	29:5	3:1	+/+	22:12	3:1	+/+	NR	
112-0864	+/+	33:0	1:0	+/+	NR		+/+	NR		+/+	23:7	3:1
112-0757	+/+	33:0	1:0	+/+	28:6	3:1	+/+	NR		+/+	NR	
112-0451	+/+	33:0	1:0	+/+	NR		+/+	21:12	3:1	+/+	NR	
120-2252	+/+	32:0	1:0	+/+	NR		+/+	NR		+/+	26:4	3:1
120-1733	−/+	31:0	1:0	+/+	22:12	3:1	+/+	20:13	3:1	+/+	NR	
120-1151	+/+	30:0	1:0	+/+	28:6	3:1	+/+	26:7	3:1	+/+	NR	
120-0942	+/+	32:0	1:0	+/+	NR		+/+	33:0	1:0	+/+	30:0	1:0
120-0629	+/+	32:0	1:0	+/+	34:0	1:0	+/+	33:0	1:0	+/+	NR	
152-1540	+/−	21:15	1:1	+/+	NR			NR		+/−	19:11	1:1
152-0954	−/+	17:19	1:1	+/+	20:10	3:1	+/+	NR		+/−	16:14	1:1
154-1111	+/+	30:0	1:0	+/+	NR		+/+	34:0	1:0		NR	
154-0974	+/+	30:0	1:0	+/+	NR		+/+	34:0	1:0	+/+	NR	
154-0366	+/+	30:0	1:0	+/+	NR		+/+	34:0	1:0		NR	

that heterozygous parents (+/−) were used in these crosses.

In the 13 crosses shown in Table 2, one initial parent had a corresponding band while another parent did not. In six of the crosses, a ratio of 1:1 was observed in F_1 progenies (markers UBC109-1740, UBC109-0803, UBC112-1962, UBC154-0793, UBC154-0764 and UBC154-0492). This mode of inheritance shows that one initial parent was heterozygous (+/−) while the other parent was homozygous for the recessive allele (−/−). In F_2 progenies for these markers Mendelian ratios (3:1, 1:1 or 0:1) were observed depending on the genotype of the F_1 parents (i.e. +/− or −/−). In the remaining seven crosses, when one initial parent had the band phenotype and the other parent did not (markers UBC109-0754, UBC112-0655, UBC112-0398, UBC120-0763, UBC152-0776, UBC154-0749 and UBC154-0449), all fish in F_1 progenies displayed the band. This mode of inheritance (1:0) indicates that in these crosses one initial parent was homozygous for the dominant allele (+/+) while the other parent was homozygous for the recessive allele (−/−). Because all F_1 fish were heterozygotic (+/−) for these markers, only theoretical ratios of 3:1 were observed in F_2 progenies (Table 2).

Twenty-four RAPD markers of the second category occasionally fitted theoretical simple Mendelian inheritance patterns, but did not display reproducibility in all progenies (Table 3). During scoring, an RAPD marker was regarded as non-reproducible if it failed to amplify or amplification was too weak for scoring, or did not fit consistently predicted Mendelian ratios in replicate gels. The reproducibility of these markers did not depend on whether the same or different thermal cyclers were used for replicate PCR amplifications. Sixteen markers from this category are shown in Table 3; these markers demonstrated non-reproducible results in either one or two progenies (from the four progenies investigated). There were eight markers from this category (not shown in Table 3) that could not be reliably scored in three of the four progenies analysed.

Thirteen RAPD markers of the third category had segregation ratios that differed from expected Mendelian ratios in all replicate gels in at least one progeny (F_1 or F_2). Eight markers did not fit Mendelian inheritance ratios in F_1 progenies, while five markers demonstrated non-Mendelian ratios in F_2 progenies (Table 4).

Discussion

The purpose of this study was to examine the inheritance and reproducibility of RAPD markers in two consecutive generations of common carp. Twenty RAPD markers were fully reproducible and demonstrated dominant Mendelian inheritance in two consecutive generations. These markers can now be used in genetic diversity studies in common carp.

Table 4 Observed (OBS) segregation of random amplified polymorphic DNA markers, which did not fit expected (EXP) Mendelian ratios in some progenies (bold)

	P	F_1		$F_1 \times F_1/1$	F_2-1		$F_1 \times F_1/2$	F_2-2		$F_1 \times F_1/3$	F_2-3	
Marker	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**	♀/♂	**OBS**	**EXP**
109-2125	+/−	**31:1**	**1:0/1:1**	+/+	21:10	3:1	+/+	24:10	3:1	+/+	19:11	3:1
109-1366	−/+	**29:3**	**1:0/1:1**	+/−	14:17	1:1	+/+	NR		−/+	18:12	1:1
109-1125	+/−	16:16	1:1	+/−	16:15	1:1	+/+	24:10	3:1	−/−	**1:29**	**0:1**
112-1262	+/−	17:15	1:1	+/−	NR		+/+	**9:23**	**3:1**		NR	
120-0821	−/−	**30:0**	**0:1**	+/+	NR		+/+	**12:21**		+/+	NR	
120-0697	+/+	32:0	1:0	+/+	**31:3**	**1:0/3:1**	+/+	NR		+/+	30:0	1:0
120-0403	+/+	**31:1**	**1:0/3:1**	+/+	34:0	1:0	+/+	33:0	1:0		NR	
152-1844	+/+	**30:2**	**1:0/3:1**	+/+	NR			NR		+/+	25:5	3:1
152-1403	+/−	**27:5**	**1:0/1:1**	+/+	NR			NR		+/+	23:7	3:1
152-1182	−/−	**36:0**	**0:1**	+/+	NR		+/+	25:9	3:1	+/+	**17:13**	**3:1**
154-2111	+/−	16:15	1:1	+/+	**16:19**	**3:1**	+/+	NR		+/+	NR	
154-1647	+/+	30:0	1:0	−/+	NR		+/+	**32:2**	**1:0/3:1**		NR	
154-0847	−/+	**26:4**	**1:0/1:1**	−/−	0:34	0:1		NR			NR	

Twenty-four RAPD markers demonstrated Mendelian inheritance but were non-reproducible in some progenies. The question of the reproducibility of RAPD markers has been repeatedly raised in previous studies with the RAPD marker system (Stott *et al.* 1997; Bardakci 2001; Ali *et al.* 2004; Liu & Cordes 2004). The low reproducibility of the RAPD technique could be due to the low annealing temperature used in the PCR amplification (Liu & Cordes 2004). The rate of amplification might be improved if PCR reaction conditions are re-optimized for individual RAPD loci. Amplification can also be improved by optimization of PCR reaction conditions, such as the concentration of template DNA, as well as magnesium and primer concentrations (Aitchitt, Thangavelu & Mantell 1998; Liu, Li, Argue & Dunham 1999).

Finally, 13 markers in the present study displayed ratios in some progenies that did not fit simple Mendelian inheritance patterns. Based on analysis of the data presented in Table 4, failure to amplify RAPD makers in some individual fish samples may have led to segregations that differed from Mendelian ratios. Six markers (UBC109-2125, UBC109-1366, UBC120-0697, UBC120-0403, UBC152-1844 and UBC154-1647) demonstrated several (one to three) 'no band' individuals in progenies produced by '+ × +' or '+ × −' crosses, which distorted theoretical ratio 1:0 (all fish have band). These 'no band' profiles could have resulted from poor DNA amplification.

The appearance of non-parental bands might be caused either by the absence of amplification in parental RAPD profiles or by the occurrence of artefact bands in the progeny. Two non-parental bands were observed in F_1 progenies for markers UBC120-0821 and UBC152-1182 (Table 4). Because these bands were observed in all F_1 individuals and these markers were transmitted to F_2, it is very probable that these bands resulted from the absence of amplification in RAPD profiles in at least one parent used in crosses. A non-parental band was also observed for marker UBC109-1125 in progeny F_2-3 (Table 4). Apparently, it was an artefact band because it was observed in only one fish, yielding a distorted segregation of 1:29 instead of theoretical ratio 0:1 (no fish have band). The most probable explanation for the appearance of artefact bands is contamination of DNA samples with pathogens or cross-contamination with other fish samples. The non-specificity of the RAPD primers could have resulted in the appearance of artefact bands by amplification of foreign DNA in the sample.

The unexpected segregation patterns for markers UBC152-1403 and UBC154-0847 (Table 4) in F_1 can also be explained by the absence of amplification in parental RAPD profiles. In these progenies, the observed segregations (27:5 and 26:4 for markers UBC152-1403 and UBC154-0847 respectively) differed from theoretical ratios 1:0 or 1:1 for '+ × −' crosses but did not differ statistically from Mendelian ratio 3:1 for cross '+ × +'. It is reasonable to suppose that the 'false-negative' parents used in these crosses appeared due to the absence of amplification.

In conclusion the non-reproducibility of RAPD markers and distorted ratios may be caused by the absence of amplification, poor amplification or by the appearance of artefact bands. Random amplified

polymorphic DNA markers with poor reproducibility and non-Mendelian inheritance can lead to misinterpretations of data in population studies, resulting in errors in the estimation of genetic diversity within and between individual populations. Therefore, it is recommended to first identify the set of reproducible RAPD markers that demonstrate Mendelian inheritance before application of the RAPD technique in population studies.

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