RAPD POLYMORPHISM IN CHINCHILLA BREEDING STOCKS

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INTRODUCTION

Increasing importance of Chinchilla breeding in Hungary set the need for detailed genetic study of different stocks. Wide choice of different DNA polymorphism systems are accepted in population characterizations and comparisons. There is no much information available about chinchilla genome, therefore molecular genetic marker investigation is rather limited as a comparative tool in evaluation of different stocks. Fortunately some methods, which are not species-specific are in use, which can still provide an appropriate tool for investigation of populations of a rarely studied species. RAPD (random amplified polymorphic DNA) investigation is one of these kind of methods (Williams *et al*., 1990). Arbitrarily chosen 10-mer DNA sequences and some of their combinations were tested against the chinchilla genomic DNA and chosen the most informative ones (generating at least 2-3 variable fragments) for genotyping 6 chinchilla breeding stocks. Altogether 20 RAPD markers were used for characterization.

MATERIAL AND METHODS

Separately bred 6 chinchilla parents stocks were taken into investigation :

Lines	Male	Female	Altogether
D1	21	19	40
D2	13	12	25
B2	8	4	12
B4	7	5	12
B5	8	8	16
B6	8	7	15
Altogether	65	55	120

Standard DNA isolation method was used for processing small earlobe slices (protein digestion and salting out). 80-100 ng DNA was taken into PCR reaction using Dynazyme DNA polymerase (Finnzymes) in appropriate buffer in 15 μl volume. 26 RAPD primers of AP Biotech and Operon alone and in combinations were tested for variable fragments. RAPD-PCR protocol (Williams *et al*., 1990) was carried out in 9700 GeneAmp thermocycler 4 min 95 ^{o}C denaturation followed by 45 cycles of 15 sec. 95 ^{o}C, 1 min. 36 ^{o}C and 2 min. 72 ^{o}C extension. PCR products were separated on 1.5 % agarose gel with ethidium-bromide. Pictures were captured and analysed by Vilber Lourmat gel documentation system.

RESULTS AND DISCUSSION

28 RAPD primer and combination were tested and proved to be monomorphic or polymorphic to different extent. 1-4 variable fragments were found in the case of informative primers. The most suitable 7 primers (6 single and 1 pair) providing 20 markers were chosen for the population studies.

Primers are found informative and used in genotyping :

AB1 : CCGTCGGTAG	AB12 : CCTGTACCGA
AB2 : GGAAACCCCT	AB13 : CCTACCGTGG
AB4 : GGCACGCGTT	AB14 : AAGTGCGACC
AB9 : GGGCGACTAC	AB19 : ACACCGATGG

Frequency of variable fragments are shown in table 1. Based on the polymorphic RAPD markers, level of genetic similarity was estimated according to Nei (table 2.) and proportion of polymorphic loci was calculated as well as estimated level of heterozygosity (tables 3-4.) (Hajós-Novák, 1999).

Table 1. Frequency of the positive allele at 20 RAPD markers in different chinchilla stocks

Primer	bp	D1	D2	B2	B4	B5	B6	Primer	bp	D1	D2	B2	B4	B5	B6
AB9	**1050**	0.68	0.76	0.42	0.42	0.63	0.33	**AB14**	**1150**	0.45	0.64	0.00	0.50	0.13	0.67
AB9	**910**	0.63	0.96	0.17	0.58	0.81	0.47	**AB14**	**460**	0.98	0.96	0.83	0.55	0.94	0.73
AB9	**350**	0.40	0.24	0.83	0.92	0.44	1.00	**AB14**	**420**	0.05	0.20	0.17	0.18	0.50	0.00
AB12	**1800**	0.90	0.64	0.92	0.92	0.75	1.00	**AB19**	**1300**	0.85	0.48	1.00	0.42	0.94	0.87
AB12	**1160**	0.25	0.36	0.42	0.33	0.19	0.00	**AB19**	**1200**	0.73	0.80	0.92	0.50	0.63	0.60
AB12	**690**	0.53	0.96	0.58	0.83	0.94	0.20	**AB19**	**920**	0.05	0.00	0.00	0.00	0.00	0.00
AB1	**1160**	0.75	0.60	0.50	0.67	0.25	0.20	**AB19**	**650**	0.73	0.52	0.58	0.25	0.50	0.80
AB1	**1130**	0.93	0.80	1.00	1.00	1.00	1.00	**AB2**	**1140**	0.23	0.68	0.00	0.50	0.25	0.20
AB1	**1050**	0.58	0.00	0.33	0.17	0.63	0.13	**AB2**	**780**	0.80	0.04	0.25	0.50	0.81	0.73
AB1	**950**	0.13	0.16	0.08	0.42	0.19	0.13	**AB4/13**	**1020**	0.35	0.36	0.50	0.83	0.88	0.33

Table 2. Nei's estimate of similarity

	D1	D2	B2	B4	B5	B6
D1	-					
D2	0.91	-				
B2	0.94	0.85	-			
B4	0.90	0.89	0.91	-		
B5	0.94	0.90	0.91	0.91	-	
B6	0.92	0.86	0.94	0.92	0.86	-

Table 3. Proportion of polymorphic loci on the base of the 20 markers

	%
D1	100
D2	90
B2	75
B4	90
B5	90
B6	70

Table 4. Estimated level of heterozygosity

	heterozygosity
D1	0,36
D2	0,32
B2	0,26
B4	0,34
B5	0,33
B6	0,24

Stock specific fragments were not found, but some of the fragments seemed to be monomorphic in some lines with exclusively presence or absence being suitable for stock identification. The twenty markers were easily scored and reproducible during investigations and suitable for future stock comparisons, identifications.

REFERENCES

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