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研究报告

水貂自咬症病因 RAPD 遗传分析

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摘 要: 自咬症是危害笼养水貂的一种慢性疾病, 造成水貂自咬创伤而影响其生长发育和毛皮质量。文中从遗传基因角度探讨水貂自咬症的发病原因在国内外尚属首次, 采用 RAPD 技术分别对正常水貂和自咬水貂样本进行了分子水平的遗传结构分析。从 100 个随机引物中筛选出 26 个重复性好的标记引物, 对 60 只水貂群体(健康与患病)进行随机扩增多态 DNA(RAPD)标记研究。结果表明, 26 个引物扩增出 105 条带, 其中 29 条带呈现多态, 多态率为 27.62%。不同引物扩增出的 DNA 片段在健康与患病水貂群体中的分布频率不同。水貂群体间相似系数为 0.8471, 遗传距离(变异)指数为 0.1529。引物 S356(序列为 CTGCTTAGGG)扩增出健康与患病水貂互不相同的条带, 如在患病水貂群体中扩增出的 1000 bp 左右的 DNA 片段, 可初步作为区分健康和患病水貂群体的分子遗传标记, 逐渐剔除自咬水貂个体, 达到净化水貂群的目的, 减少水貂饲养业的经济损失, 为今后水貂的分子育种及其疾病的预防提供一定的理论依据。

关键词: 水貂, 自咬症, RAPD, 发病原因, 分子遗传标记

RAPD Genetic Analysis on Etiological Factor of Mink Self-biting Disease

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Abstract: Self-biting is a chronic disease, which cause wound to take effect on mink growth and pelt quality. In this study, we firstly adopted RAPD (random amplification polymorphism DNA) technique based on the reproducible 26 polymorphism primers screened from 100 random primers to analyze hereditary constitution of the samples from healthy minks and self-biting minks, respectively, at molecular level to aim to discuss the causes of self-biting. The results showed that 29 straps showed polymorphism among amplified 105 straps, of which the polymorphism rate is 27.62%. Between healthy and sick mink groups, the amplified DNA fragment through different primers indicated different distribution frequency. The similarity coefficient of mink groups is 0.8471 and genetic distance (variation) index is 0.1529. Through primer S356 (whose sequence is CTGCTTAGGG), we amplified different straps between healthy and sick mink. The amplified 1000 bp DNA fragment in the sick mink groups can preliminarily serve as molecular genetic label to distinguish from healthy and sick mink groups to gradually remove the mink individual of self-biting, achieve to purify mink groups and reduce economy loss of mink breeding industry. This work provide theoretical basis for further study on molecular breeding and disease prevention of mink.

Keywords: mink, self-biting disease, RAPD (random amplification polymorphism DNA), etiological factor, molecular genetic labeling

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RAPD is a kind of DNA polymorphism detective technology and was developed by Williams[1] and Welsh[2] laboratory in 1990. It has many characteristics such as convenience, shortcut, economy, sensitivity and so on. It manifests large potential and superiority in classifying and appraising biological populations. Because RAPD technology can survey polymorphism on the whole genome, if bolting suitable primer we can find distinctive species, stock or colonial RAPD labeling, and then carry out species or groups appraising. Moreover, RAPD analyses DNA variation of genetic material, and is never influenced by environmental condition and individual development stage. Above all we can conveniently analyze DNA multiplicity without any molecular biological documents about the species genome[3]. Self-biting is a chronic disease and jeopardizes mink in the cage[4–9]. Now people do not understand etiological factor of self-biting, scholars from domestic or foreign country have many different opinions[10–16]. We adopted RAPD technology to analyze hereditary constitution of molecular level on normal mink and self-biting mink samples and demonstrated the etiological factors of self-biting whether is genetic factor or not. So it may provide a theoretical basis for further study on molecular breeding and disease prevention of mink.

1 Materials and methods

1.1 Experimental samples

We took muscles on leg of normal mink (30) and self-biting mink (30) samples randomly through asepsis ways from the same mink field in Dalian. Then put them into EP tubes with 75% alcohol (easy to transport) and kept in –20°C.

1.2 Extraction and detection of genome DNA

Genome DNA was extracted by phenol-chloroform extracting way[17], then we detected DNA purity and density through agarose gel electrophoresis and violet absorption spectrometry, and diluted DNA to 50 ng/μL, –20°C conservation.

1.3 Preparation of cistern DNA

Take 30 DNA templates (each 30 μL) randomly from 60 minks (normal and sick), mingle and dilute them to constitute a DNA cistern, and ensure density of each DNA sample is 50 ng/μL. Then we took 10 μL genomic DNA from 30 normal minks and 30 sick minks respectively, and mixed them together for usage in RAPD labeling, then we can bolt primer of different mark.

1.4 Bolting random primer

100 S series random primers were bought from Shanghai sangon Biological Engineering Technology Company. Then we amplified cistern DNA using 100 primers and bolted primer of polymorphism abundance, strap clear and master tape repetitiveness well. We amplified two cistern DNA (health and disease) by using the bolted primers, each reaction has two repetitions at least, and set up negative control not containing lamellar[18,19].

1.5 Random amplification reaction system and conditional dominance

PCR reaction system: 10×buffer (2.0 μL), 25 mmol/ L $MgCl_2$ (1.0 μL), 2.5 mmol/L dNTP each 1.5 μL, random primer (1.0 μL), DNA product (1.0 μL), Taq DNA polymerase (0.2 μL), ddH_2O.

Reaction condition: 95°C 4 min; 94°C 50 s, 36°C 1 min, 72°C 1 min, for 35 circles; 72°C 10 min, 4°C for conservation. The PCR products were detected by 1.2% agarose gel electrophoresis.

1.6 Statistical analysis

Never consider to fallibility band (tenuity and impossible repeating stripe in the same condition) and frequency below 5% stripe in the whole samples. According to formula of Nei and Li, then we calculated RAPD signed fragment share degree (F) from healthy minks and self-biting minks, that is to say genetic resemblance and genetic diversity (genetic distance) index number (D), $F=2N_{XY}/(Nx+N_Y)$, $D=1-F$. N_{XY} represents all the fragment numbers of identical molecular weight of DNA amplified production in the X group and Y group. N_X and N_Y represent DNA amplification production of healthy group and sick group respectively[20].

2 Results

2.1 Detection of DNA density and purity

The results in Table 1(different genome DNA density results) showed that DNA contents of the individual minks (health and disease) were more than 100 ng/μL. This content was enough to carry out PCR reaction and further RAPD analysis. Through agarose gel electrophoresis (Fig. 1) detection, the genomic DNA was integrated without degradation phenomenon.

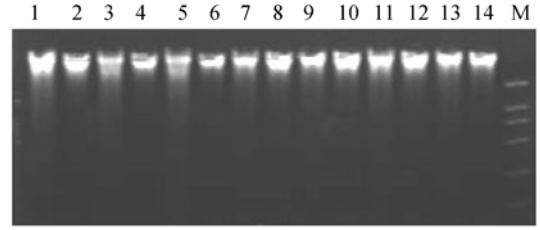

Fig. 1 Electrophoresis of individual mink genomic DNA
1–14: extracted genomic DNA; M: DL2000 marker

2.2 Result of bolting primer

We bolted 26 amplified primers from 100 random primers. The amplified production mostly intervene between 200–1500bp (Fig. 2). Then we carried out RAPD analysis of mixed DNA of health and disease mink, among of them, amplified productions of eight

Table 1 The genome DNA content determination result of different mink individual

DNA sample (health)	OD_{260}	OD_{280}	OD_{260}/OD_{280}	DNA content/(ng/μL)	DNA sample (disease)	OD_{260}	OD_{280}	OD_{260}/OD_{280}	DNA content /(ng/μL)
1	0.134	0.076	1.752	670	31	0.066	0.040	1.650	330
2	0.160	0.089	1.798	800	32	0.186	0.100	1.860	930
3	0.175	0.100	1.750	875	33	0.038	0.022	1.727	190
4	0.069	0.037	1.864	345	34	0.064	0.039	1.641	320
5	0.033	0.019	1.736	165	35	0.082	0.048	1.708	410
6	0.052	0.029	1.793	260	36	0.073	0.043	1.698	365
7	0.081	0.047	1.724	405	37	0.141	0.073	1.931	705
8	0.080	0.047	1.702	400	38	0.172	0.094	1.819	860
9	0.060	0.035	1.714	300	39	0.095	0.055	1.727	475
10	0.105	0.060	1.750	525	40	0.100	0.057	1.754	500
11	0.036	0.021	1.714	180	41	0.051	0.030	1.700	255
12	0.044	0.025	1.760	220	42	0.067	0.039	1.718	335
13	0.041	0.024	1.708	205	43	0.054	0.031	1.742	270
14	0.099	0.058	1.707	495	44	0.146	0.085	1.718	730
15	0.074	0.041	1.805	370	45	0.059	0.034	1.735	295
16	0.102	0.059	1.729	510	46	0.027	0.015	1.800	135
17	0.077	0.045	1.711	385	47	0.115	0.067	1.716	575
18	0.077	0.044	1.750	385	48	0.032	0.018	1.778	160
19	0.151	0.086	1.756	755	49	0.098	0.057	1.719	490
20	0.113	0.066	1.712	565	50	0.123	0.072	1.708	615
21	0.065	0.037	1.757	325	51	0.022	0.012	1.833	110
22	0.100	0.057	1.754	500	52	0.051	0.029	1.759	255
23	0.072	0.042	1.714	360	53	0.043	0.025	1.720	215
24	0.143	0.083	1.723	715	54	0.054	0.031	1.742	270
25	0.067	0.036	1.861	335	55	0.140	0.079	1.772	700
26	0.111	0.064	1.734	555	56	0.029	0.016	1.813	145
27	0.183	0.102	1.794	915	57	0.150	0.080	1.875	750
28	0.090	0.052	1.731	450	58	0.032	0.018	1.778	160
29	0.078	0.043	1.696	390	59	0.097	0.054	1.794	485
30	0.096	0.054	1.778	480	60	0.081	0.046	1.761	405

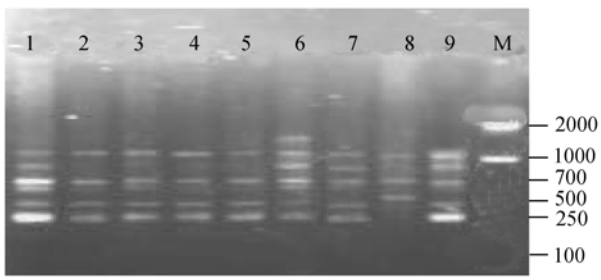

Fig. 2 PCR products of mink mixed DNA(health and disease) with random primer

1–9: random amplified result of cisterna DNA; M: DL2000 marker

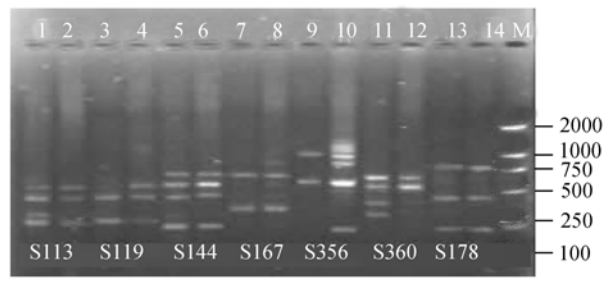

Fig. 3 PCR products of two groups (health and disease) cisterna mink DNA with random primer

1, 3, 5, 7, 9, 11, 13: cisterna DNA from healthy mink; 2, 4, 6, 8, 10, 12: cisterna DNA form mink with self-biting disease

Table 2 Random amplification results of 26 primers in the two DNA cisterna

Primers	Sequences	Health group (strip)	Disease group (strip)	Mark number (strip)	Primers	Sequences	Health group (strip)	Disease group (strip)	Mark number (strip)
S102	TCGGACGTGA	3	3	0	S140	GGTCTAGAGG	3	4	1
S103	AGACGTCCAC	6	4	2	S143	CCAGATGCAC	4	4	0
S104	GGAAGTCGCC	4	4	0	S144	GTGACATGCC	2	3	1
S105	AGTCGTCCCC	3	4	1	S145	TCAGGGAGGT	3	3	0
S111	CTTCCGCAGT	1	1	0	S167	CAGCGACAAG	6	6	1
S112	ACGCGCATGT	2	1	1	S176	TCTCCGCCCT	5	4	1
S113	GACGCCACAC	3	1	2	S178	TGCCCAGCCT	4	3	1
S114	ACCAGGTTGG	2	2	0	S346	TCGTTCCGCA	3	2	1
S118	GAATCGGCCA	4	5	1	S349	TGAGCCTCAC	5	3	2
S119	CTGACCAGCC	3	3	0	S356	CTGCTTAGGG	5	7	3
S120	GGGAGACATC	3	3	1	S358	TGGTCGCAGA	0	3	3
S130	GGAAGCTTGG	3	2	1	S359	GGACACCACT	2	2	0
S139	CCTCTAGACC	6	2	4	S360	AAGCGGCCTC	4	2	2

primers manifested single state, eighteen primers manifested polymorphism (Fig. 3). Bolting 26 primers amplification band number in the middle of 0 to 7, together record 105 amplification strip, polymorphism fragments were 29 strips (polymorphism rate 27.62%). The amplification results with different primers were listed in Table 2.

2.3 Reproducibility analysis

We bolted 26 random primers and carried on 3 repeat amplification protocol in the same condition respectively. The results showed the reproducibility of the straps amplified by 11 primers was good (S103, S113, S119, S139, S144, S145, S167, S176, S356, S359, and S360, respectively) (Figs. 4 and 5). Primer S356 amplified different straps of healthy and sick mink, for example, the 1000 bp strap only existed in sick mink. It was significant difference and similarity of amplification bands of primer S356 (Fig. 6).

2.4 Statistical analysis

Between healthy and sick mink groups, the amplified DNA fragment through different primers indicated different disposition frequency. Mink groups similarity coefficient is 0.8471, genetic distance (variation) index is 0.1529 (Table 3).

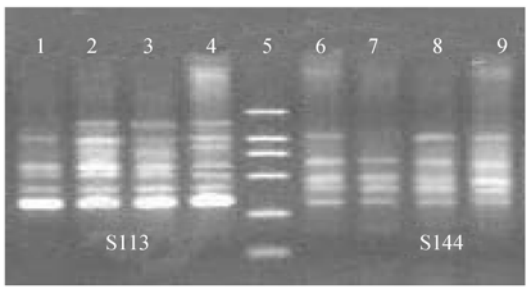

Fig. 4 Amplification result of reproducibility with random primers S113, S144

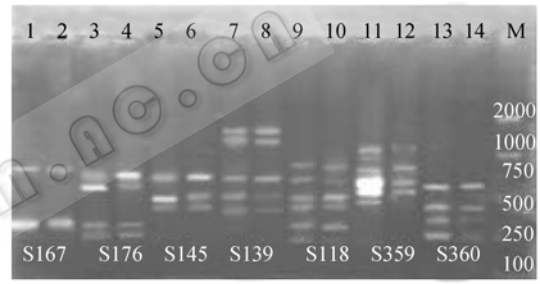

Fig. 5 Amplification results of reproducibility with random primer

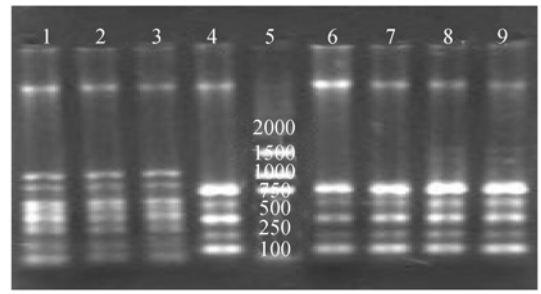

Fig. 6 PCR products from mink (healthy and sick) with primer S356

1–3: amplification result of self-biting cisterna DNA; 5: DL 2000 marker; 4, 6–9: amplification result of healthy cisterna DNA

3 Discussion

3.1 Genetic analysis on healthy mink and sick mink

Self-biting of mink lead to descending level of mink skin, every year productions of mink skin are about fifty million all over the world. Descended production of skin are about two million to three million because of self-biting[21]. Etiological factor of self-biting have many different opinions from domestic or foreign

Table 3 RAPD fragment similarity and genetic index of variability in the healthy and sick minks

Health group mark number (Nx)	Disease group mark number (Ny)	Identical mark number genetic (Nxy)	Similarity coefficient (F)	Genetic index of variability (D)
89	81	72	0.8471	0.1529

country scholars, Therefore classification of self-biting has not been definited. Merch veterinary handbook classified self-biting into questionary disease[22–23]. Pathogeny of mink self-biting whether is caused by genetic gene or not. We have never seen any detailed reports about difference individual gene between self-biting to healthy mink. In this experiment, we adopted RAPD molecular labeling method to carryout genetic analysis of mink self-biting. Results indicated that some genes had obvious differences between the healthy and sick mink. In this experiment straps were amplified by primer S356 had obvious commonness and difference. Primer S356 amplified different straps of healthy and sick mink, for example, the 1000bp strap only existed in sick mink, so the trap may be the characteristic strap of sick mink. In order to check stability of polymorphism sign, we will increase sample size to validate effect on self-biting of these marks. Meanwhile, we will search for more marks to provide scientific base for molecule breeding of mink and gradually reject self-biting mink in order to reach purified mink and decrease economic loss of mink breeding industry.

3.2 PCR amplification and RAPD labeling

RAPD is a kind of method to analyze unknown genome DNA. And take polymorphism DNA fragment through amplifying random primer as molecule mark, this method is useful to analyze genetic diversity of man, animal, plant and germ and appraise stock[24–29]. RAPD mark cover integral chromosome, the results can objectively manifestation the degree of genetic homology. Therefore, it can directly reflect on similarity information among of the research object[30]. Compare to other heredity detective methods, RAPD never require foreseeing series information of genome and design primer. After set up this reaction we find detective speed is faster than microsatellite. The result never be effected by living environment, sampling position and developmental stage of sampling object, detect many polymorphic site, convenient operation and shortcut. Moreover primer in random, amount can almost increase arbitrarily. Primer is cheap and easy to obtain, so it is suitable to research mark and fingerprinting. It is extremely fit to adopt bulk primers to analyze polymorphism of genome. There is obviously shortage because its primer randomness and amplified site are indeterminateness in the whole genome, we can not definite amplification site[31, 32]. If apply large primers to find some characteristic RAPD mark, not only provide molecule base of genetic aspect but also combine with other mark methods to clone purpose gene and mark-assisted selection. Provide reference for hereditarily breeding of mink and application in the medical domain. RAPD amplification need 10 bp random primers, PCR reaction permit to have 1–4 bp misalliance in the 5′ extreme of primer. It is the main reason to lead to lower repetitiveness of RAPD technique[33]. In order to make result have more reliability, it is important to choose stable primer. In this experiment, we selected 26 primers through many times definition and strictly control consistent reaction. So mark polymorphism rate was high and well polymorphism. The studies indicated that RAPD technology must choose suitable primer and strictly control consistent reactive condition. We will have ability to acquire well results.

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