DOI: 10.2478/v10083-012-0013-1

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXX (2) SECTIO EE 2012

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Identification of DNA sequences affecting chinchilla (C*hinchilla lanigera* Molina, 1782) behaviour

Identyfikacja sekwencji DNA wpływających na behawior szynszyli
(*Chinchilla lanigera* Molina, 1782)

Summary. The aim of the study was to determine the relationship between the types of chinchilla behaviour and different images of band patterns obtained using the RAPD-PCR technique. The sound-move test allowed classification of the animals into confident, reserved and indifferent ones. The results of the pilot study based on markers OPA07 and OPA12 suggest the existence of DNA regions containing nucleotide sequences that determine chinchilla behaviour. It is advisable that further analyses should be performed aimed at discovering sequences of genetic material that may determine the traits studied. This will facilitate using the band pattern as an additional criterion, beside lineage information, in animal selection.

Key words: chinchilla, RAPD-PCR, behaviour

INTRODUCTION

One of the common traits in selection of livestock animal species is their mild temper, manifesting itself in the ability of quelling fear, and a low level of aggressiveness towards man. As stated by Pavlova *et al.* [2007], animal reactions to extreme treatment and negative emotions have become a particular value. When exposed to an aversive stimulus, animals exhibit passive behaviour strategies. Observations of the behaviour of breeding chinchillas have been held for a relatively short time. Dzierżanowska-Góryń

and Kowalczyk [2005] have found that maintenance of animals in adverse environmental conditions exerted a detrimental effect on their psyche; it may lead to self-mutilation, fur biting, and decreased appetite. Each type of psychological disturbance in chinchilla may be manifested by excessive aggression, reserve, and timidity in response to a new stimulus. Therefore, aggressive or timid animals should be eliminated from breeding. Such animals are characterized by lower growth rates; they have difficulty in reproduction and rearing their kits. Occasionally, young chinchillas are cannibalized or destroyed by mothers that are exposed to stress.

The choice of the behavioural test is a key element in determination of the type of chinchilla behaviour [Ślaska and Jeżewska-Witkowska 2008a]. The aforementioned study results allow a conclusion that, instead of upgrading of the sufficiently favourable environmental conditions on chinchilla farms, selection of animals with the best reproduction welfare can be performed in conditions established by humans. Such management may bring measurable benefits.

In breeding of fur-bearing animals, genetic markers are used for origin control, precise determination of maternal half-siblings in one litter, construction of genetic maps, and identification of genetic distance and genetic differences and similarities between different groups of individuals. By identification of genetic variability, molecular markers may provide valuable information about the population and its structure, the level of gene flow, phylogenetic relationships, historical biogreographical models, and analysis of origin and relatedness. The results of the pilot study based on the use of RAPD markers [Ślaska and Jeżewska-Witkowska 2008b] suggest the existence of DNA regions containing nucleotide sequences that are responsible for both the body weight and the total conformation in chinchillas. Therefore, RAPD markers may be used as an additional criterion for formation of genetic groups in selection of mating animals.

The aim of the study was to determine the relationship between chinchilla behaviour and the different images of band patterns established using the RAPD technique.

MATERIAL AND METHODS

The study material consisted of chinchilla var. standard (33 females and 15 males) born on one of the breeding farms in eastern Poland in the years 2006-2007. The animals were maintained in standard bedding cages lit by artificial light with 12-h day light regime and at temperature ca. 18°C (+/-2°C). A sound-move test described by Ślaska and Jeżewska-Witkowska [2008a] was performed in order to assess the chinchilla temper. The choice of the test from among several behavioural tests applied in chinchillas was prompted by the results of the study on the reproduction welfare in chinchilla [Ślaska and Jeżewska-Witkowska 2008a].

The observations were conducted between the 4^{th} and 8^{th} hour of the daylight by a person unknown to the chinchillas, since the sanitary and veterinary treatment was provided by the farm owner, which evoked particular behaviour of individual animals towards their breeder.

Based on the observations of chinchilla behaviour in the behavioural test, females exhibiting a similar type of response were classified into the following groups:

I. Confident, curious animals – those that were not scared by the sound of the door opening; they would immediately approach the observer and leant out of the cage within 20 seconds; they did not exhibit any reaction that might imply the feeling of anxiety towards the stranger;

II. Reserved, curious animals – this group comprised animals that showed their interest within 60 seconds after the beginning of the observation, i.e. individuals that approached the object or open door within 20-60 seconds; these individuals came up to the door but did not lean out of it; they were fully alert and ready to escape from a possible external stimulus;

III. Indifferent animals – those that did not react to the sound-move stimulus; they usually sat on the shelf or in the cage corner and did not exhibit special interest in the external environment.

DNA was isolated with the use of the QIAamp DNA Blood Mini Kit (QIAGEN) from the hair follicles of the experimental chinchilla. The PCR reaction followed quantitative and qualitative assessment of DNA samples. The reaction mixture (sample volume 30 μl) contained 80 ng DNA; 3 μl PCR buffer, 4.2 μl Q solution, 200 μM of each nucleotide, 0.2 μM of the arbitrary primer, 25 mM $MgCl_2$, 1 U Taq polymerase. Two primers were used for the amplification reaction: OPA07 (5'→3'GAAACGGGTG) and OPA12 (5'→3'TCGGCGATAG) (Proligo Primers and Probes).

The markers were selected on the basis of their polymorphism described in the study of Ślaska et al. [2008]. The amplification reaction (PTC-225 DNA Engine Tetrad thermocycler) consisted in initial denaturation (94°C, 5 min.); 46 cycles of: denaturation (94°C, 1 min.), primer binding (36°C, 2 min.), lengthening of DNA strands (72°C, 1 min.); terminal lengthening of primers (72°C, 10 min.) and cooling to temp. 4°C. The RAPD-PCR products were fractionated in 2% agarose gel (using a bromophenol-blue loading solution). The gels were analysed in UV light (Transiluminator) and archived. GeneRuler 50bp DNA Ladder oraz GeneRuler 100bp DNA Ladder Plus (Fermentas) were used as markers of the DNA fragment size.

The probability of appearance of specific DNA fragments related to the temper of the individuals revealed by the sound-move test was verified by the one-way analysis of variance [SAS Institute INC 2000]. Based on the presence (1) or absence (0) of the band of each of the markers studied in the agarose gel, the probability of its presence in animals exhibiting particular behaviour types was determined. This was aimed to identify bands that might contain DNA sequences determining chinchilla behaviour. The amplified DNA fragments with the length of 1050, 1200 and 1500 base pairs (in the case of OPA07) and 600 bp (OPA12) were excluded from the analysis, since they were found in all the animals tested.

These pilot investigations aimed at indication of specific DNA bands that could contain genes or regulatory regions of genes affecting particular animal behaviour types are preliminary. They will instigate further research focused on SCAR – Sequence Characterized Amplified Regions – markers, originating from RAPD markers [Cushwa and Medrano 1996, Gu et al. 1999]. They will reveal DNA regions that may contain the above-mentioned nucleotide sequences.

RESULTS AND DISCUSSION

RAPD polymorphism results from presence or absence of particular bands generated by the primer, the cause of which can be the difference in the nucleotide sequence in the primer-binding region. Mutation in only one nucleotide site results in lack of amplification. Presence or absence of the product may also originate from insertion or deletion of the fragment between two conservative primer-binding sites. Investigations of *Chinchilla lanigera* genome using RAPD-PCR markers had previously been carried out in chinchillas [Hidas *et al*. 2002, Ślaska and Jeżewska-Witkowska 2008b, Ślaska *et al.* 2008]. Ślaska *et al*. [2008] evaluated the usefulness of RAPD-PCR markers in genetic analyses in chinchillas; among the 10 genetic markers, the most polymorphic DNA fragments were generated by, *inter alia*, OPA07 and OPA12.

In the chinchilla group studied, confident animals constituted 35.42% (47.06% males, 52.94% females), reserved animals – 35.42% (29.41% males, 70.59% females), and indifferent animals – 29.17% (14.29 % males, 85.71% females) of all the individuals tested. No aggressive behaviour was reported among the animals observed on the breeding farm.

The absence of chinchilla characterized by aggressive behaviour on the experimental farm may be explained by the fact that, unlike mild-tempered animals, aggressive individuals are eliminated from the breeding due to their lower growth rates and poorer reproduction performance and, in particular, rearing the kits [Dzierżanowska-Góryń and Kowalczyk 2005, Gacek 2002].

The investigations of the type of chinchilla behaviour based on markers OPA07 and OPA12 are of a pilot nature. Based on the analyses performed, the probability of presence of particular bands related to the type of chinchilla behaviour was determined using the sound-move test and the OPA07 (Fig. 1, Tab. 1) and OPA12 markers (Fig. 2, Tab. 2).

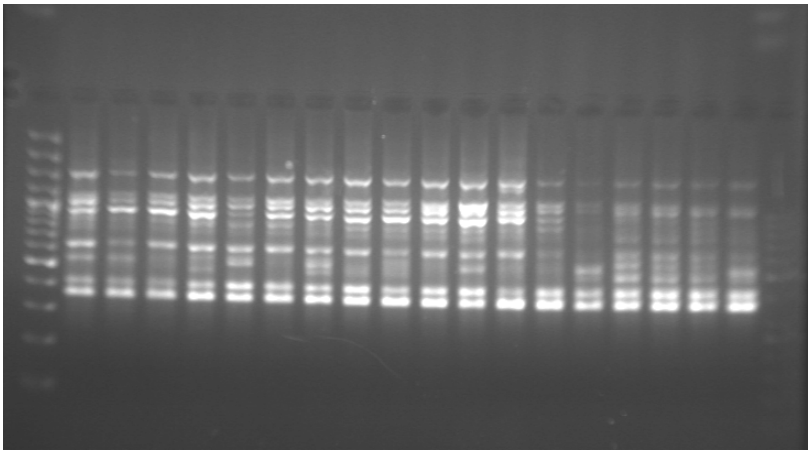

Fig. 1. Electrophoregram of the RAPD-PCR analysis in chinchilla using primer OPA 07 (the first lane on the right and on the left – the marker of size)
Rys. 1. Elektroforegram analizy RAPD-PCR u szynszyli z wykorzystaniem startera OPA 07 (pierwsza ścieżka od prawej i lewej – marker wielkości)

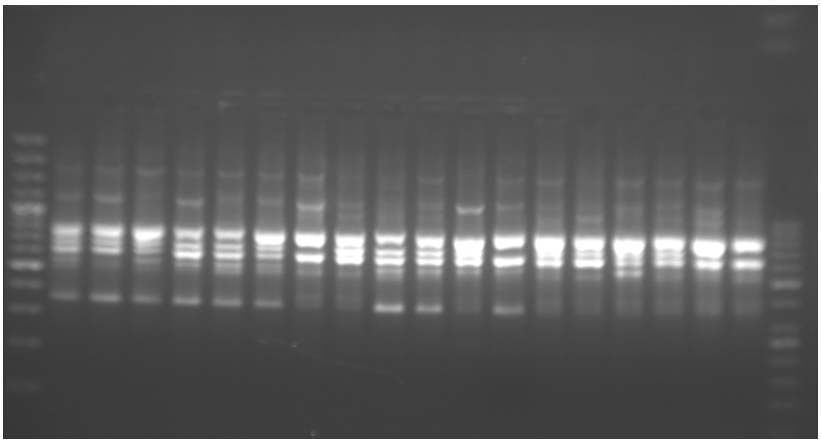

Fig. 2. Electrophoregram of the RAPD-PCR analysis in chinchilla using primer OPA 12 (the first lane one the right and on the left – the marker of size)
Rys. 2. Elektroforegram analizy RAPD-PCR u szynszyli z wykorzystaniem startera OPA 12 (pierwsza ścieżka od prawej i lewej – marker wielkości)

The study does not include classification of the animals into the male and female groups within the particular behaviour types as no significant differences in the probability of presence of individual bands were observed in these animal groups. Moreover, in the case of both the markers analyzed, none of the bands obtained was characteristic for one gender exclusively.

Table 1. Probability (P*) of the presence of particular OPA07 marker bands in relation to the type of behaviour
Tabela 1. Prawdopodobieństwo (P*) występowania poszczególnych prążków markera OPA07 w zależności od typu zachowania

Size of amplified DNA fragments (bp)** Wielkości amplifikowanych fragmentów DNA (pz)**	Type of behaviour – Typ zachowania					
	confident ufne		reserved powściągliwe		indifferent obojętne	
	P*	se	P*	se	P*	se
250	0.118	0.059	0.000	0.059	0.071	0.065
300	0.412	0.120	0.294	0.120	0.429	0.132
350	0.059[b]	0.101	0.294	0.101	0.429[a]	0.111
400	1.000	0.047	1.000	0.047	0.857	0.052
500	0.471	0.115	0.765	0.115	0.714	0.126
550	0.000[b]	0.065	0.059	0.065	0.214[a]	0.072
600	0.470	0.121	0.706	0.121	0.500	0.133
700	0.941[a]	0.099	0.765	0.099	0.500[b]	0.109
800	0.941[a]	0.097	0.765	0.097	0.643[b]	0.107
900	0.765	0.110	0.765	0.110	0.643	0.121

** – Approximate values – Wartości przybliżone.
[a,b] – The values in the lines marked with various letters differ significantly at $p \leq 0.05$ – Wartości w wierszach oznaczone różnymi literami różnią się między sobą istotnie przy $p \leq 0,05$.

As regards primer OPA07 (Tab. 1), special attention should be paid to the 350, 550, 700 and 800 bp bands. The probability of appearance of 700 and 800 bp bands was significantly highest in the group of the confident animals, compared to the indifferent chinchillas. Statistically significant differences concerning the probability of presence of 350 and 550 bp bands were also found between the groups mentioned above. Among the indifferent chinchillas, the probability of presence of the aforementioned fragments was higher than that in the other animal groups. Therefore, it is possible that the DNA fragments investigated contain nucleotide sequences that can exert an effect of the type of chinchilla behaviour.

While analyzing the data presented in Table 2, attention should be paid to the significant differences concerning the presence of DNA fragments in the particular chinchilla groups characterized by different behaviour. The 400 bp band exhibited the highest probability of presence in the group of the confident chinchillas, in comparison with the other animals. 800 and 1750 bp DNA fragments exhibited the highest probability of appearance in the group of the indifferent chinchillas.

Table 2. Probability (P*) of the presence of particular OPA12 marker bands in relation to the type of behaviour
Tabela 2. Prawdopodobieństwo (P*) występowania poszczególnych prążków markera OPA12 w zależności od typu zachowania

Size of amplified DNA fragments (bp)** Wielkości amplifikowanych fragmentów DNA (pz)**	Type of behaviour – Typ zachowania					
	confident ufne		reserved powściągliwe		indifferent obojętne	
	P*	se	P*	se	P*	se
350	0.709	0.100	0.882	0.100	0.786	0.110
400	0.647[a]	0.119	0.471	0.119	0.286[b]	0.131
500	0.882	0.081	0.941	0.081	0.786	0.089
550	0.588	0.116	0.765	0.116	0.643	0.128
700	0.588	0.114	0.706	0.114	0.786	0.126
800	0.647[b]	0.106	0.647 [b]	0.106	0.929[a]	0.117
900	0.353	0.101	0.235	0.101	0.071	0.111
1050	0.471	0.115	0.294	0.115	0.214	0.126
1200	0.412	0.124	0.471	0.124	0.571	0.136
1500	0.235	0.106	0.176	0.106	0.357	0.117
1750	0.000[b]	0.086	0.176	0.086	0.357[a]	0.094
2000	0.118	0.082	0.176	0.082	0.071	0.090

** – Approximate values – Wartości przybliżone.
[a,b] – The values in the lines marked with various letters differ significantly at $p \leq 0.05$ – Wartości w wierszach oznaczone różnymi literami różnią się między sobą istotnie przy $p \leq 0,05$.

The results obtained by other authors [Dzierżanowska-Góryń and Kowalczyk 2005, Rozempolska-Rucińska *et al.* 2007] indicate that breeders should carry out selection to obtain confident animals. This is related to the fact that mild-tempered females are characterized by higher reproduction rates, in comparison to timid or aggressive females. However, according to other authors [Gacek 2002, Ślaska and Jeżewska-Witkowska 2008a], extremely mild-tempered females are not desirable for breeding due to lower fecundity and lower rearing performance of the offspring.

Investigations conducted by Gu *et al*. [1999] have demonstrated that a single band with a specific size obtained by the RAPD reaction may be composed of more than one DNA fragment of the same size, but with a different nucleotide sequence. However, Espinasa and Borowsky [1998] reported a necessity to confirm the homology of the bands obtained in investigations of phylogenetically remote species. In the present study, confirmation of homology seems unnecessary, since the investigation involved one species. Therefore, the assumption that the anonymous DNA fragments with the same size obtained in the profiles of the individual animals represent homologous loci is valid.

Previous investigations on a chinchilla group [Ślaska and Jeżewska-Witkowska 2008b] allowed identification of the relationship between the body weight and total confirmation and different images of band patterns obtained with the RAPD technique. The results imply existence of DNA sequences in particular bands determining the performance traits. It was concluded based on the estimation of chinchilla reproduction welfare [Ślaska and Jeżewska-Witkowska 2008a] that females classified by the sound-move test as reserved were characterized by the highest fecundity and number of weaned kit per litter. The present results (Tab. 1) reveal that the 250 bp sequence (OPA07) was absent in the reserved chinchillas. The probability of the presence of the 600 bp band was also higher in the aforementioned group, compared to that in the confident and indifferent animals. In the case of marker OPA12 (Tab. 2), the significant differences in the presence of 400, 800, and 1750 bp DNA fragments indicate a necessity of continuation of the study. Further analyses (SCAR) of the DNA fragments are advisable due to the possibility of identification of the sequences contained therein. It cannot be excluded that within each DNA fragments of the analyzed markers there are nucleotide sequences that can significantly affect the type of chinchilla behaviour.

The analysis results obtained will facilitate further observation focused on SCAR – *Sequence Characterized Amplified Regions* – markers originating from RAPD markers [Cushwa and Medrano 1996, Gu *et al*. 1999].

CONCLUSIONS

1. Individual DNA fragments of markers OPA07 and OPA12 should be used for further analyses aimed at identification of the nucleotide sequences contained therein.

2. It cannot be excluded that, after additional genetic analyses, markers OPA 07 and OPA12 will be used as an additional criterion in creation of genetic animal groups with respect to their behaviour.

3. The study results may facilitate future use of the individual DNA fragments as supplementation to lineage data for genetic preservation of individual types of behaviour.

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Streszczenie. Celem pracy było określenie związku pomiędzy typami zachowania szynszyli a różnym obrazem wzorów prążkowych szynszyli ustalonych techniką RAPD-PCR. Z wykorzystaniem testu dźwiękowo-ruchowego wyodrębniono zwierzęta ufne, powściągliwe i obojętne. Otrzymane w pracy wyniki pilotażowych badań z wykorzystaniem markerów OPA07 i OPA12 sugerują istnienie rejonów DNA, w których mogą znajdować się sekwencje nukleotydowe warunkujące zachowanie szynszyli. Przeprowadzenie dalszych analiz, mających na celu ustalenie sekwencji materiału genetycznego mogącego determinować badane w pracy cechy jest celowe. Umożliwi to wykorzystanie wzoru prążkowego, obok informacji rodowodowych, jako dodatkowego kryterium selekcji zwierząt.

Słowa kluczowe: szynszyla, RAPD-PCR, behawior