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Bi-paternal Litter in Finn Raccoon (*Nyctereutes procyonoides* Gray 1834) Detected by Polymorphic DNA Markers

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The present paper demonstrates novel results confirming the phenomenon of multipaternity in Finn raccoons. The purpose of the study was to affirm the occurrence of bi-paternal litters in Finn raccoons using polymorphic microsatellite markers. The study was carried out on 37 Finn raccoon females, males that mated with them and the resulting offspring (176 individuals). The offspring came from females mating with two or three different males during one estrus. Parentage testing of 15 microsatellites was used in the present study. Based on genetic analysis including the control of the origin of the Finn raccoons, it was observed that in the case of double-mating with two different males, as well as triple-mating with three different males, there were 47.06% and 54.55%, respectively, of maternal half-sibs, i.e., animals born in one litter but with different fathers. Paternity was wrongly ascribed in 18.2% of the offspring in the examined Finn raccoon population. The conducted research indicates a necessity to check the origin of young animals selected for breeding stock in Finn raccoon breeding farms.

Key words: Breeding biology, multiple mating, polyandry, microsatellites, *Nyctereutes procyonoides*.

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The Finn raccoon (*Nyctereutes procyonoides*) is one of the fur-bearing animals belonging to the family Canidae. The profitability of Finn raccoon farming mainly depends on the fertility of females, which are seasonally monoestrous. That is why a female, as a rule, mates with not only one but several males twice or three times during one estrus, which lasts from 2 to 6 days (VALTONEN & MÄKELÄ 1980). It is difficult to determine which male is the biological father of the obtained offspring, however, this information has principle significance for breeding work in Finn raccoon farms and selection of animals for breeding stock. In breeding practice (pedigree data), the father of the offspring is the last male (the second or the third) the female mated with. Such an approach can lead to small effectiveness of selection because there is no certainty if the attributed father of the litter is their actual biological father. Moreover, during the selection of reproductive pairs, this can lead to mating closely related animals and the uncontrolled appearance of inbreeding depression with all its negative results, such as decreased fertility of animals. If a female mated with different males during one estrus is considered, is only one male the biological father of the offspring? Natural populations of some species should also be considered in this respect. Investigations regarding multiple mating were conducted in reference to different species of animals (KEIL & SACHSER 1998; KLEMME *et al.* 2007; KYLE *et al.* 2007; RATKIEWICZ & BORKOWSKA 2000; FISHER *et al.* 2006). Many types of animal behaviour are designed to rear the largest number of offspring. An example of this is promiscuity in some species, the effect of which can be multipaternity. Having information about the occurrence of maternal half-sibs (LEWIS *et al.* 2005; KEIL & SACHSER 1998; RATKIEWICZ & BORKOWSKA 2000), it seems purposeful to undertake investigations in Finn raccoons.

The purpose of the study was to confirm the occurrence of bi-paternal litters in Finn raccoons using polymorphic microsatellite markers.

Material and Methods

The research was conducted on 37 Finn raccoon females, males that mated with them and also their offspring (176 individuals). The investigated progeny descended from females mating with two different males (17 litters) and with three males (11 litters) during one estrus. The control group included 9 males and 9 females as well as their offspring, resulting from a single mating. RFinn raccoons maintained at a Polish breeding farm in the years 2002-2004 were used for the analysis.

Blood samples were collected from the animals using sterile test-glasses (Medlab) with K_2EDTA as an anticoagulant. Genomic DNA was isolated using QIAamp DNA Blood Mini Kit (QIAGEN). Parentage testing of 15 microsatellites according to ŚLASKA *et al.* (2007) was used in the present study. All primers as well as conditions of PCR (*Polymerase Chain Reaction*) amplification of microsatellite sequences as in ŚLASKA *et al.* (2005) was performed. Allele identification of microsatellite loci was conducted by capillary electrophoresis performed in a ABI PRISM^{TM} 3100-Avant Genetic Analyzer using: 3100-AVANT ABI PRISM DATA COLLECTION and GENE MAPPER SOFTWARE 3.5. The Windows-based computer program CERVUS, according to MARSHALL *et al.* (1998), was used to perform parentage analysis using data from all types of codominant markers.

Results and Discussion

The available literature does not provide data on the occurrence of maternal half-sibs in a single litter in Finn raccoons. On the basis of the DNA marker genotypes, the utility of paternal tests was assessed in the control group consisting of females mated with a single male during a single estrus and their offspring. Genotype compatibility between the offspring and the parents was observed in the control group, based on the STR markers. Next, biological fathers were determined for the offspring born by the females which mated with two or three different males during one estrus.

Genetic analysis including the Finn raccoons descent control showed that 52.9% of the litters from the double mating (two different males) and 45.45% of all the litters from the triple mating (three different males) descended from one of the mating males (Table 1). On average, in 50.81% of all the litters descending from the double or triple matings, we observed maternal half-sibs, i.e., animals born in the same litter but having different fathers. This occurred in 8 litters of double-mated and 6 litters of triple-mated females (Table 1).

Research on multiple mating has been carried out on various animal species, including rodents such as *Galea musteloides* (KEIL & SACHSER 1998), *Clethrionomys glareolus* (KLEMME *et al.* 2007; RATKIEWICZ and BORKOWSKA 2000) and hoary marmots (*Marmota caligata*) (KYLE *et al.* 2007), as well as on an Australian marsupial *Antechinus stuartii* (FISHER *et al.* 2006). However, in the accessible literature there is no data about the occurrence of maternal half-sibs in one litter of *Nyctereutes procyonoides*. In this paper we present novel results that confirm the occurrence of multi-paternity in Finn raccoons.

In fur bearing animal breeding it is the last mating male that is regarded as the father of the offspring from multi-mating a single female during one estrus. Our results show that this approach may lead to the occurrence of significant pedigree discrepancies. In the case of 18.2% of the offspring, paternity was wrongly ascribed in the investigated population of Finn raccoons. It thus seems obvious that the phenomenon of multipaternity indicates improper work in breeding farms at the stage of animal selection for the breeding stock, where only pedigree data documentation is taken into account. In the light of the these considerations it should be emphasized that apart from inaccurate assessment of animal breeding value, misled paternal ascription may result in inbreeding depression in the stock with all its negative effects, primarily in lowered fertility (HOLT *et al.* 2005).

Table 1

Characteristics of the investigated population of Finn raccoons with respect to the number of matings

Number of matings*	Full sibs in the litter		Half-sibs in the litter		Total	
	Number of litters	Percentage participation	Number of litters	Percentage participation	Number of litters	Percentage participation
Single mating	9	100	–	–	9	100
Double mating	9	52.94	8	47.06	17	100
Treble mating	5	45.45	6	54.55	11	100

*concerns mating with a female during one estrus.

NEFF & PITCHER (2005) discussed the biological mechanisms for acquiring and promoting the genetic quality of offspring. During breeding, the promotion of well conditioned progeny may take place. Depending on the time of occurrence NEFF & PITCHER (2005) divided this into 3 stages: (1) precopulatory (mate choice); (2) postcopulatory, prefertilization (sperm utilization) and (3) postcopulatory, postfertilization (differential investment). At each stage, promotion of fit offspring may occur. NEFF and PITCHER (2005) provided a (re)definition of genetic value that comprises "good genes" and "compatible genes" while presenting the difference between genetic value and genetic benefit. Genetic value affects the individual fitness of an animal, which can be defined as individual lifetime reproductive success, LRS, including both survivorship and breeding success. Consequently, both components are significant in determining genetic value (KOKKO *et al.* 2002; HUNT *et al.* 2004). According to NEFF & PITCHER (2005), the female aims at improving the fitness of her offspring, which in unfavourable circumstances can worsen; this implies that genetic values are underestimated by the female in favour of genetic benefit.

In numerous animal species females selectively choose males at the time of mating (KOKKO *et al.* 2002). Through this type of selection the female may increase the genetic benefit due to better offspring fitness. In Finn raccoon breeding farms the precopulatory choice of a male is not possible, thus the female loses the chance of promoting the genetic value of the offspring. LEWIS *et al.* (2005) observed that females can confine paternity success of particular mating males by subsequent mating with a male that is more preferred. It can be concluded from their research that during a triple mating the paternity success of the first two males is diminished to the advantage of the third one. Thus, female fertility does not increase, but the proportions between paternity success of particular males change. This phenomenon may result from processes which take place in the female organism after insemination. When a female mates with several males during one estrus, sperm precedence is determined by the processes of sperm storage and usage (LEWIS *et al.* 2005).

FISHER *et al.* (2006) reported that in females mating with several males polyandry may improve offspring survivorship. They also observed that in multiple mating between closely related males and females polyandry reduces the relative number of inbred progeny and improves female lifetime fitness in nature. The above mentioned facts indicate numerous biological reasons for the occurrence of multipaternity in animals.

The results of the conducted research confirmed the hypothesis of the occurrence of bi-paternal litter phenomenon in Finn raccoons. Using polymorphic microsatellite markers, the authors proved that the offspring from one litter from females that mated with several males during one estrus may come from different fathers. Therefore, it is essential that the father of each animal which is assigned for breeding stock and which comes from a double- or triple-mating should be ascribed in an objective and reliable way (ŚLASKA *et al.* 2007).

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