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Selection for behavior and hemopoiesis in American mink (*Neovison vison*)

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ABSTRACT

It has been known that selective breeding of animals for behavior associated with domestication changes a variety of physiological traits including response to stress and hypothalamo–pituitary–adrenocortical system activity. The aim of our research was to find association between behavioral type and differential leukocyte counts as an indicator of homeostasis activity. We used farm-raised 5-month-old minks (*Neovison vison*) that had been selective bred for 17 generations on the basis of behavior toward human. After selection, minks were divided into the following groups: aggressive (from −4 to −1), "domestic" (from +1 to +5). Blood leukocytes were analyzed in 5-month-old minks (n = 105) of different behavior lines: aggressive (−1, −2), "domestic" (+1 and from +3 to +5), fearful, and minks never subjected to selective breeding (control). We found strong effects of selection for behavior of minks on differential blood leukocyte count. There were differences between behavior types in differential leukocyte counts. Neutrophils and eosinophils were the leukocyte types the most sensitive to selection for behavior. The counts of eosinophils in peripheral blood in "domestic" (+3, +5) minks were higher than in control and aggressive (−2) minks. The percentage of neutrophils was higher in the most aggressive minks (−2) in comparison to "domestic" minks. We have concluded that differences found in leukocytes profile are the result of neuroendocrine system modification.

Introduction

The American mink (*Neovison vison*), a North American native semiaquatic generalist predator, has expanded its range globally as a result of their breeding in fur farms (Dunstone, 1993; Bonesi and Palazon, 2007). First attempts to keep wild individuals under human care, thus starting the domestication process, have reportedly taken place around 1866 (Shackelford, 1949, 1984), when trappers in Canada began to keep and breed wild mink in enclosures. Farm mink after domestication differ from their wild counterparts in fur color, skull dimensions, brain, body size, and temperament (Belliveau et al., 1999; Kruska, 1996; Kruska and Sidorovich, 2003). Domesticated mink are bred to be larger, with higher reproductive success and less aggressive than wild ones. Fearfulness and fear-induced aggression in mink may be beneficial in a natural context but may be detrimental to the animal's welfare in the captive environment. Therefore, one of the first selected traits in the history of mink domestication was tame behavior to humans (Trapezov, 2000).

The investigation of the effects of domestication provides insight into evolution problems and is of practical interest. One of the first selection experiments for domestic behavior was conducted with silver fox (*Vulpes vulpes*) at the Institute of Cytology and Genetics, Novosibirsk, Russia (Belyaev, 1979). Belyaev (1979) supposed that selection of silver fox for behavior in response to human interference can be a pivotal factor of domestication. Long-term selection of farm-bred mink (*Neovison vison*) for tame and aggressive defensive reaction toward man has been carried out at the Institute's experimental farm since the 1980s. Tame and aggressive lines of standard (+/+) minks differing in their defensive response toward humans were developed using a breeding protocol designed to study the effects of destabilizing selection (Klotchkov et al., 1998). As revealed in studies using silver foxes and minks, behavior-controlling genes

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have a broad pleiotropic effect. Selection of silver foxes and minks for low aggression and tameness modifies numerous regulatory processes and leads to a variety of morphological, behavioral, and physiological traits. Trapezov (2013) and Wilkins et al. (2014) note certain traits of domestication in mammals: depigmentation, floppy ears, shorter muzzles, smaller teeth, docility, smaller brain or cranial capacity, more frequent estrous cycles, neotenous (juvenile) behavior, curly tails.

The influence of selection on the activity of regulatory systems controlling homeostasis has been demonstrated for numerous animal models. Selection for low aggressiveness to humans leads to a change in behavioral manner and is accompanied by genetically determined changes of the neuroendocrine system (Klotchkov et al., 1998). A result of selection for domestic behavior in minks is alteration of the hypothalamo–pituitary–adrenocortical system (HPAS) activity (Trapezov, 2000) and levels of serotonin and other monoamines in the mink brain (Nikulina et al., 1985). Animals selected over several generations for tameness showed lower basal and stress-induced hypothalamo–pituitary–adrenocortical activity, reached sexual maturity earlier, and were easier to mate than more fearful animals (Nikula et al., 2000; Malmkvist, 2001; Pedersen et al., 2002).

There is a need to study the association of behavioral type and differential leukocyte counts as the indicator of homeostasis activity. According to some authors, the effects of HPAS under stress include elevated plasma corticosteroids, reduced T- and B-lymphocyte proliferation and neutrophil phagocytosis, increased neutrophil/lymphocyte ratio, as well as leukocyte adhesiveness/aggregation (Irwin et al., 1990; Song et al., 1994). Since domestication changes neuroendocrine system activity, we can expect animals within distinct behavior categories to show discrepancy in differential leukocyte count. As revealed in previous works by Davis et al. (2008), the counting of white blood cells from blood smears (leukocyte profiles) can be used to assess stress and welfare in animals. They concluded that this given method can provide a reliable assessment for stress in all vertebrate taxa.

The aim of the present study was to research differential leukocyte counts in American mink with different behavior types under prolonged experimental domestication.

Materials and methods

Experimental animals

Experiments were performed with standard dark brown (+/+) minks at the experimental farm facility of the Institute of Cytology and Genetics (Novosibirsk, Russia). The animals were tested for defensive reaction to man only once a year at 5-month age (September) by one person (Trapezov) throughout the selection period (17 generations). The “hand catch test” was applied for this purpose. Both males and females were tested for defensive reaction toward man during an entire day, with breaks for the morning and evening feeding. The experimenter opened the cage where the tested animal was kept, slowly reached for the animal, and tried to catch it with a hand protected with a special mitten.

The technique allowed discrimination among 3 types of behavior toward man: aggressive, fearful, and “domestic” (tame). To study minks of different behavioral categories, we followed the classification of Trapezov (1987). Mink with fearful behavior tried to avoid contact with man as much as possible, and when the experimenter tried to catch them, they ran about the cage in panic, shrieking. This behavior was assigned score “0.”

Aggressive behavior varied qualitatively, which allowed scoring it on a 4-point scale as follows.

Score 1 (fearful response toward humans)

When attempts were made to catch the mink, it rapidly retreated, hid in its nest box, gaped and bared its teeth, cried shrilly or hissed; its posture was tense, showing severe emotional stress.

Score 2 (attack from the nest box)

When attempts were made to catch the animal, it jumped to the entrance of the nest box and hid there to attack the experimenter’s gloved hand from the box and bit it fiercely.

Score 3 (active attack outside the shelter)

When attempts were made to catch the mink, it immediately attacked the experimenter’s hand instead of hiding.

Score 4 (onset of attack in response to a human approaching)

Even before the test began, that is, before the experimenter opened the cage, the mink loudly shrieked, ran about the cage, and gnawed the bars.

Animals with domesticated behavior did not show any signs of fear or aggression on contact with man, which allowed the experimenters to work barehanded. On the whole, the domestic behavior was defined as absence of fear or defense reaction toward man.

The expression of domesticated behavior also varied qualitatively, making it possible to score it using a 5-point scale as follows.

Score +1 (exploratory responses)

The mink calmly responded to the experimenter’s stretched hand and showed an exploratory response, sniffing the hand and quivering the vibrissae.

Score +2 (calm response to contact with human hand)

The mink accepted the forced contact with the experimenter’s hand and did not retreat, allowing the hand to touch its face, chest, and paws.

Score +3 (active contact on the part of the animal)

When the experimenter opened the cage, the mink got up leaning against the open door and reached out with its snout to the human hand. Within the cage, the mink actively sniffed about the hand, examined it, and often leant against the hand with its paws.

Score +4 (the mink allowed any part of its body to be touched)

The animal displayed an active exploratory response, examined the experimenter’s hands, played with them, but resisted all attempts to be handled.

Score +5 (the mink allowed humans to handle it)

These individuals displayed the most pronounced domesticated behavior, allowing the experimenters to handle them without showing any signs of fear or aggression.

Selective breeding-specific patterns of handler-directed behavior have been ongoing for 17 generations based on the results of annual tests. The study reported here was performed on 5-month-old male and female minks ($n = 105$) selected for “domestic,” aggressive, and fearful behavior. The control group included minks of the same age that had never been subjected to selective breeding. Animals were chosen at random from the population of minks scoring +1, +3, +4, +5 (“domestic” group), and from the population scoring −1, −2 (aggressive group). The sample did not include +2 and −3, −4 scored minks as animals within these groups were too few. Table 1 shows the numbers of animals in each group and their sex distribution.

All animals were homed in regulation cages on the same farm and given diet and water. All the experiments were conducted