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# Epigenetic Variations in Nonmetric Skull Traits in American Mink (*Neogale vison* Schreber, Carnivora, Mustelidae) Strains after Selecting for Defensive Behavioral Characteristics

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**Abstract**—The occurrence of discrete nonmetric threshold traits (NTTs) of the axial skull and mandible was studied among strains of aggressive and tame American minks (*Neogale vison* Schreber 1777), obtained after selecting for characters of defensive behavior at an experimental fur farm. Non-selected caged and wild Canadian minks were taken as control groups. After culling the NTTs with invariant frequencies, unclear topologies, single, rare (<5%), and high-frequency (>95%), three versions in the array of traits were used: "expanded" (50 traits), allowing for their connection with sex and size; "restricted" (30), excluding such a connection; and "combined" by sex (50), where the frequencies of males are only taken for sex-related traits. An assessment of the mean measures of divergence (*MMD*) in terms of the frequency of occurrence of NTT phenes in all versions revealed significant differences between the strains, as well as both control groups. In the first version, the differences between the sexes were most pronounced, compared to those between the strains in the second and third ones. In all versions, aggressive and tame minks differed to the maximum degree, whereas the caged non-selected individuals occupied an intermediate position. When comparing samples, wild Canadian minks are the closest to caged non-selected minks, the divergence between aggressive and tame exceeding the difference between caged and wild. Canonical analysis of the principal components characterizing the manifestation of individual phenetical compositions for a constrained set of 30 NTTs (with lower environmental and greater hereditary conditionality) revealed the same intergroup variations as on the basis of *MMD*. The effect of selection based on characters of defensive behavior for 16–17 generations was found to be accompanied by a greater differentiation of aggressive and tame American minks than wild and caged ones as a result of their almost century-long isolation of the latter in fur farms. The values of the indices of epigenetic variability (*EV*) and the volume of within-group morphospace (*Vm*) characterizing the degree of destabilization of development, are significantly higher in tame minks than in aggressive ones. The results are in good agreement with Belyaev's theory of destabilizing selection and indirectly indicate a high rate of epigenetic changes in experimental strains of the American mink, this accounting for the high adaptive potential of this invasive species during its range expansion in Eurasia.

**Keywords:** mink, epigenetic polymorphism, phenetic distances, domestication
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## INTRODUCTION

The response to selection for defensive behavior characteristics is closely related to the emergence of typical genetic and morphogenetic effects during animal domestication (Belyaev, 1979a, 1979b; Trut, 1981; Trapezov, 1987; Belyaev and Trut, 1989; Singh et al., 2017). Many authors have demonstrated the high rate of morphogenetic rearrangements observed during the domestication of dogs, silver foxes, American minks, brown rats, and other species (Drake and Klingenberg, 2010; Kharlamova et al., 2000; Singh et al., 2017; Trut et al., 2021). In the American mink, morphological changes appeared already at the initial stage of the experiment after several generations of selection for defensive behavior characters (Kharlamova et al., 2000). Such rapid responses of domesticated species to selection for behavioral features likely cannot be due solely to the effects of selection on random genome mutations affecting morphogenesis (Kukekova et al., 2018). Moreover, the degree of characteristic morphogenetic rearrangements that develop over a relatively small number of generations is significant (Singh et al., 2017).

It can be assumed that another likely factor in the rapid emergence of a selective response may be changes in morphogenesis, which are caused by stress-induced epigenetic processes (DNA methylation, transposition of mobile genomic elements, etc.), the leading role of which in microevolution has been increasingly discussed in recent decades (Jablonka

and Raz, 2009; Burggren, 2016; Donelan et al., 2020). A similar mechanism of genetic, epigenetic, and ethological rearrangements associated with changes in their morphogenesis in experimental animals during selection for defensive behavior features is in good agreement with the theory of destabilizing selection proposed by Belyaev (Belyaev, 1979a, 1979b).

Morphometric differences were previously identified when comparing strains of aggressive and tame American minks (Kharlamova et al., 2000), obtained on an experimental fur farm at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, based on selection for defensive behavior characters. However, many aspects remained unexplored, including interstrain epigenetic differences and manifestations of epigenetic variability marked by nonmetric threshold traits (NTTs) of the skeleton (Grüneberg, 1963; Berry and Searle, 1963; Sjøvold, 1977; Hartman, 1980; Ulevičius et al., 2001; Wójcik et al., 2007).

The use of discrete nonmetric skeletal traits in genetic comparisons was started in the mid-1950s by English geneticists of the Grüneberg school (Grüneberg, 1952, 1952a, 1963; Deol and Truslove, 1957; Grewal, 1962) on linear mice and continued on natural populations of mammals (Berry and Searle, 1963; Sjøvold, 1977; Hartman, 1980; Andersen and Wiig, 1982; Ansorge, 2001). Once manifested in the phenotype, nonmetric threshold traits (NTTs) vary like quantitative traits and are under the control of genetic and epigenetic factors (Grüneberg, 1952b) and can mark the genetic and epigenetic specificity of mammalian lineages and populations (Grüneberg, 1963). Grewal (1962), using the C57BL strain with known genealogy as an example, estimated the rate of genetic divergence of its subsrains for a complex of nonmetric traits. High heritability of some nonmetric traits of the skull and teeth has been established in humans (Berry, A.C. and Berry, R.J., 1967; Berry, 1978). Berry and Searle (1963) defined the discrete manifestation of skeletal NTTs as "epigenetic polymorphism," clearly understanding the epigenetic nature of the discreteness of bilateral structures and relying on the epigenetic landscape model of Waddington (1957). In linear mice, it was shown that NTT frequencies are highly resistant to the effects of environmental stressors during development (Bauchau, 1988; Vasil'ev and Vasil'eva, 2009). Recently, NTTs have been used to assess epigenetic divergence and epigenetic variability indirectly (Wójcik et al., 2007; Ansorge et al., 2009; Vasil'ev and Vasil'eva, 2009; Korablyov et al., 2018, 2020).

Previously, Vasil'ev et al. (2004) identified significant differences in the cranial NTT complex between experimental strains of aggressive and tame silver foxes. Korablyov et al. (2018) discovered geographic variability in NTTs in invasive wild populations of the American mink in European Russia and their differences from farmed mink. Hence, it could be expected that similar differences would also be found between strains of aggressive and tame American mink on the experimental fur farm at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences. Therefore, it was of interest to compare experimental American mink strains formed through selection (16–17 generations) for the NTT complex. It is important to determine whether selection for defensive behavioral characteristics influenced differences in the expression of NTT phenes across strains. It is necessary to evaluate the ratio of differences in phene frequencies between strains of aggressive and tame caged mink, as well as control caged non-selected and wild mink from Canada. It is necessary to determine whether there are differences in the expression of epigenetic variability and the level of stability of bilateral NTT development in the experimental mink strains.

The aim of the work is to assess the differences in the occurrence of phenes of nonmetric threshold traits of the axial skull and mandible between experimental strains of aggressive and tame American minks, as well as control caged non-selected and wild Canadian representatives of the species, taking into account the manifestation of epigenetic variability and stability of the development of bilateral morphological structures.

## MATERIALS AND METHODS

This study uzed collection craniological material from two American mink strains obtained after 16–17 generations of selection based on defensive behavior characteristics at an experimental fur farm at the Institute of Cytology and Genetics SB RAS under the supervision of O.V. Trapezov (1987, 2012). Samples of aggressive (males, AM, $n = 31$; females, AF, $n = 31$) and tame (males, TM, $n = 30$; females, TF, $n = 31$) individuals were studied. Non-selected minks (males, NM, $n = 34$; females, NF, $n = 36$) were used for comparison and served as a conventional control. All animals were represented by yearlings of similar age (seven months) obtained in the same season (November). Additionally, a sample of wild males from the natural population of American mink (CanM) aged 1+ to 3+ from the province of Alberta (Wood Buffalo Park, Conibear Lake, 1933) in Canada was studied, $n = 10$. The material from Canada was obtained by the museum of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, in the 1950s from exchanged collections with the National Museum of Canada and allows us to estimate roughly the degree of changes in the frequency of occurrence of NTT phenes in caged American minks compared to one of the wild Canadian populations.

The design of this study involved conducting the research in four stages: (1) primary search (screening) of phenes of nonmetric traits of the skull and mandible with subsequent rejection of invalid (with fuzzy topology), single, rare, with extremely high frequency, or invariant in frequency of occurrence, as well as related

to each other; (2) calculation of phenetic distances (*MMD*) by frequencies of nonmetric traits for three versions of their sets, their multidimensional non-metric scaling and cluster analysis; (3) canonical analysis of the values of principal components characterizing the individual manifestation of phenocompositions by a restricted set of NTTs in mink samples; (4) assessment of epigenetic variability indices (*EV*) by a restricted set of bilateral nonmetric traits, as well as calculation of the volumes of intragroup morphospaces (*Vm*), indirectly characterizing the degree of destabilization of the manifestation of phenocompositions in samples.

In the first stage, we planned to form three working versions of the NTT sets: "expanded," allowing for significant correlations with sex and size; "restricted," excluding such traits; and "combined," in which the frequencies of combined male and female samples were used for traits unrelated to sex, but only the frequencies of male samples were used for traits significantly related to sex. The use of traits in the second and third versions was aimed at reducing the environmental effects of trophism, hormonal levels, and sexual size dimorphism and enhancing the share of the heritable component of differences.

During screening of axial skulls and mandibles of American minks, after a preliminary search, about 200 phenes of medial and bilateral nonmetric threshold traits were found (additional openings for blood vessels and nerves or their reduction, additional bone elements, reduction of bone fragments, etc.). Some of the identified traits coincide with ones previously described for carnivorous mammals by other authors (Sjøvold, 1977; Wiig and Andersen, 1988; Glushkova and Korablyov, 1997; Ulevičius et al., 2001; Vasil'ev et al., 2004; Ansorge et al., 2009; Monakhov, 2010; Ranyuk and Monakhov, 2011). Since a large number of phenes is difficult not only to classify, but also to use in multivariate analysis (the number of traits should not exceed the number of individuals studied in the sample), it was decided to use 80 NTTs for further analysis with some reserve (taking into account their upcoming partial culling) (Fig. 1, Fig. A7, Tables A4, A5). Latin abbreviations of phene names are indicated in the legend to Fig. 1, and the complete list of Russian-language descriptions and abbreviated Latin names of NTTs and their states (phenes) for 80 original traits is given in the Appendix (see Tables A4, A5). In all cases, NTT was dichotomized, recording the presence or absence of a phene (threshold state).

Other features (e.g., those located inside the skull, etc.) were excluded mainly due to the increased difficulty of detection, unclear criteria for identifying phenes, broad expressivity, which did not allow for a strict identification of their threshold states, as well as their unstable topology, which hindered diagnosis. During the selection, single (e.g., a single absence of tooth m2), rare (on average, less than 5% per trait) or traits occurring with high frequency (on average, more than 95%) were also excluded. The rule of analogous comparisons (Kryštufek, 1990; Ventura and Sans-Fuentes, 1997; Wójcik et al., 2006) also excludes NTTs with invariant sample frequencies (we tested this using the multiple $\chi^2$ test).

A reclassification of the material revealed significant differences between the initial and re-evaluated frequencies of 14 NTTs among the 80 initially retained traits, due to the unclear topology of the phenes. These NTTs were categorized as invalid (see Table A5). The other 16 NTTs had invariant ("equal") frequencies, as confirmed by the $\chi^2$ test (see Table A5). The remaining 50 NTTs (Table 1, see Fig. 1) were adopted as the first expanded version of their set, which also included traits correlating with the sex and size. This set of traits made it possible to correlate the absolute range of sex and interstrain differences with the degree of differentiation of control caged and wild minks. To obtain the second version, a restricted set of NTTs (Table 1), traits significantly associated with sex and size, were removed (the condylobasal length of the skull, mm, was used as a size characteristic). Using the $\chi^2$ test, 20 NTTs were identified for which significant sex differences were found. Based on the nonparametric Spearman rank correlation analysis, 18 NTTs were identified that were significantly associated with the skull size of minks (see Tables A4, A5). As a result, we left for the second version a restricted set of 30 traits, the manifestation of which is not correlated with either sex or size (see Fig. 1, Table 1, Tables A4, A5). The third version of the combined set of 50 NTTs included the same traits as in the first version, but for those that were correlated with sex, only the frequencies of male samples were taken into account. The traits used in the three versions of the sets were classified as valid. In accordance with existing concepts (Berry, A.C. and Berry, R.J., 1967; Berry, 1978; Hartman, 1980; Ansorge et al., 2009), differences in the traits of the second and third versions of the sets, excluding the influence of sex and size on their expression, are largely due to hereditary rather than environmental factors. An assessment of the relationship between the occurrence of NTTs using Spearman's rank nonparametric correlation did not reveal coefficient values equal to or exceeding the level of weak correlation $r_S = 0.3$; i.e., all the remaining traits did not duplicate information or only slightly duplicated it.

For each sample of males and females of the compared strains of caged minks, as well as males of the wild Canadian population, the frequencies of NTT phenes were calculated, from which phenetic distances were then calculated for 50 and 30 traits. When calculating paired phenetic distances between samples, which Grewal (1962) called the "mean measure of divergence" (*MMD*), we used the formula of C.A.B. Smith as modified by Hartman (Hartman, 1980):

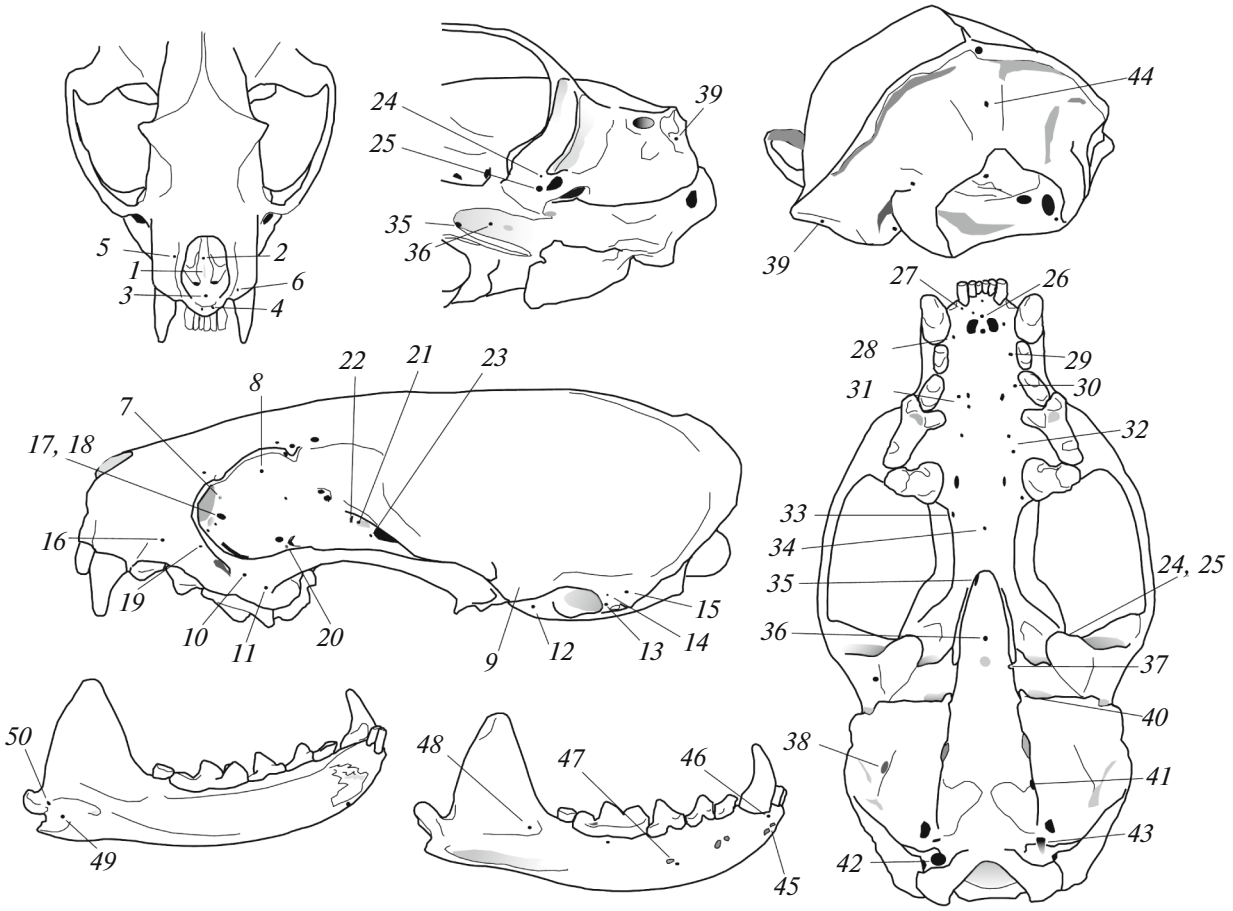


**Fig. 1.** The arrangement of nonmetric traits (nos. *1*–*50*) on the axial skull and mandible of the American mink: *1**m, FiCrns (–); *2**m, Fcrnspo; *3*m, Fpmnsme; *4**, FPmdsI2; *5*, FPmnsds; *6*, FPmnsve; *7**, FPlcim; *8*, FFran; *9**, FTm (–); *10,* FMxzgan; *11*, FMxzgpo; *12**, FPgl; *13*, FMtacla; *14*, FMtacds; *15**, FSqla; *16**, FMxca; *17**, FMxor (–); *18**, FeMxor; *19**, FIoac; *20*, FSplac; *21*, FOpac; *22,* Fspop; *23**, FFior; *24**, FRt (+); *25**, FRtac; *26*m*, FIcmeac; *27*, FAvlgIn; *28*, FAvlgC; *29*, FAvlgPm1; *30**, FAvlgPm2; *31**, FMxplmt; *32**, FPlPm3ac; *33**, FMpo; *34*m, FPlme; *35**, IsPspla; *36*m, FSpme; *37*, IsLmpt; *38**, FeTp; *39**, FStmac; *40**, PrTpan; *41**, FOctp; *42**, FeFocnif (FFCI); *43**, FJginap; *44* m, FOcme; *45**, Fmticdu; *46**, FMdcave; *47**, Fmtpodu; *48**, FMdms; *49*, FMdaglg; *50*, FMdarlg (* numbers of 30 traits not related with sex; full names and descriptions of all traits are given in the Appendix (Table A4)).

$$MMD = 1/r \sum_{i=1}^{r} \left\{ (\theta_{i1} - \theta_{i2})^2 - \left[ 1/(n_{i1} + 0.5) + 1/(n_{i2} + 0.5) \right] \right\},$$

where $\theta = 0.5 \sin^{-1}\left[1 - 2k/(n+1)\right] + 0.5 \sin^{-1}\left[1 - 2k/(n+1)\right]$ are the transformed frequencies of occurrence of phenes; $r$ is the number of traits; $k$ is the frequency of the phene; $n_{i1}$ and $n_{i2}$ are the numbers of studied sides. This modification introduces a correction for the probability of random nondetection of a trait, and therefore, zero frequency values are not used in the calculation.

The mean standard deviations (*MSD*) of phenetic distances were calculated using the Sjøvold's formula (Sjøvold, 1977):

$$MSD = 1/r \sqrt{\sum_{i=1}^{r} 2\left(1/n_{i1} + 1/n_{i2}\right)^2}.$$

For $\alpha = 0.05$, differences in *MMD* are statistically significant if they are 1.96 times greater than *MSD* (approximately for $MMD > 2\ MSD$). The measure of uniqueness of a sample (*MU*) is usually estimated as the sum of the distances of the *MMD* of a given sample with all the others (Berry, 1963; Sjøvold, 1977; Hartman, 1980). We used another method for estimating the uniqueness of a sample—the average distance of a given sample with all the others (*MMU*, the mean measure of uniqueness), which is useful when comparing the results of studies with different numbers of compared samples and traits (Vasil'ev, 2005).

To overcome possible deviations from the Euclidean metric and visualize the centroids of samples in morphospace, the non-metric multidimensional scal-

**Table 1.** Frequency (%) of 50 nonmetric skull and mandible traits used in different comparisons of males (M) and females (F) of strains of aggressive (A), non-selected (N), and tame (T) caged American minks and males of the wild population (CanM)

| Trait no. | AM $n = 62$ | AF $n = 62$ | NM $n = 68$ | NF $n = 72$ | TM $n = 60$ | TF $n = 62$ | CanM $n = 20$ |
|---|---|---|---|---|---|---|---|
| *1*m | 0.00 | 0.00 | 2.94 | 2.78 | 20.00 | 12.90 | 0.00 |
| *2*m | 61.29 | 48.39 | 55.88 | 52.78 | 36.67 | 25.81 | 30.00 |
| *3*m | 45.16 | 29.03 | 55.88 | 27.78 | 76.67 | 45.16 | 70.00 |
| *4* | 53.23 | 41.94 | 30.88 | 29.17 | 28.33 | 32.26 | 25.00 |
| *5* | 66.13 | 29.03 | 39.71 | 37.50 | 50.00 | 12.90 | 65.00 |
| *6* | 91.94 | 69.35 | 75.00 | 70.83 | 78.33 | 29.03 | 85.00 |
| *7* | 43.55 | 51.61 | 39.71 | 44.44 | 28.33 | 25.81 | 20.00 |
| *8* | 54.84 | 67.74 | 33.82 | 59.72 | 11.67 | 38.71 | 60.00 |
| *9* | 9.68 | 17.74 | 23.53 | 30.56 | 21.67 | 35.48 | 25.00 |
| *10* | 54.84 | 37.10 | 50.00 | 25.00 | 58.33 | 29.03 | 60.00 |
| *11* | 77.42 | 24.19 | 76.47 | 33.33 | 73.33 | 8.06 | 65.00 |
| *12* | 61.29 | 64.52 | 51.47 | 37.50 | 38.33 | 22.58 | 50.00 |
| *13* | 66.13 | 24.19 | 45.59 | 27.78 | 48.33 | 8.06 | 40.00 |
| *14* | 79.03 | 51.61 | 57.35 | 56.94 | 83.33 | 29.03 | 65.00 |
| *15* | 16.13 | 11.29 | 26.47 | 15.28 | 41.67 | 17.74 | 35.00 |
| *16* | 66.13 | 61.29 | 45.59 | 50.00 | 53.33 | 12.90 | 75.00 |
| *17* | 22.58 | 25.81 | 23.53 | 31.94 | 35.00 | 56.45 | 0.00 |
| *18* | 38.71 | 54.84 | 50.00 | 36.11 | 6.67 | 12.90 | 35.00 |
| *19* | 20.97 | 40.32 | 14.71 | 18.06 | 5.00 | 4.84 | 20.00 |
| *20* | 3.23 | 9.68 | 5.88 | 6.94 | 20.00 | 22.58 | 25.00 |
| *21* | 32.26 | 12.90 | 23.53 | 16.67 | 13.33 | 6.45 | 15.00 |
| *22* | 25.81 | 48.39 | 42.65 | 51.39 | 35.00 | 66.13 | 40.00 |
| *23* | 51.61 | 40.32 | 52.94 | 54.17 | 75.00 | 67.74 | 65.00 |
| *24* | 56.45 | 67.74 | 57.35 | 52.78 | 53.33 | 58.06 | 75.00 |
| *25* | 19.35 | 12.90 | 19.12 | 15.28 | 25.00 | 20.97 | 25.00 |
| *26*m | 38.71 | 35.48 | 32.35 | 22.22 | 20.00 | 20.97 | 50.00 |
| *27* | 43.55 | 12.90 | 25.00 | 29.17 | 43.33 | 4.84 | 15.00 |
| *28* | 41.94 | 27.42 | 44.12 | 30.56 | 28.33 | 9.68 | 25.00 |
| *29* | 35.48 | 12.90 | 33.82 | 15.28 | 33.33 | 19.35 | 45.00 |
| *30* | 48.39 | 32.26 | 58.82 | 43.06 | 75.00 | 56.45 | 80.00 |
| *31* | 25.81 | 16.13 | 25.00 | 18.06 | 41.67 | 43.55 | 35.00 |
| *32* | 20.97 | 22.58 | 33.82 | 37.50 | 43.33 | 38.71 | 10.00 |
| *33* | 50.00 | 48.39 | 42.65 | 40.28 | 40.00 | 33.87 | 35.00 |
| *34*m | 22.58 | 3.23 | 29.41 | 5.56 | 3.33 | 3.23 | 50.00 |
| *35* | 83.87 | 75.81 | 88.24 | 93.06 | 98.33 | 91.94 | 60.00 |
| *36*m | 6.45 | 22.58 | 32.35 | 25.00 | 43.33 | 45.16 | 30.00 |
| *37* | 72.58 | 50.00 | 77.94 | 69.44 | 65.00 | 43.55 | 40.00 |
| *38* | 8.06 | 14.52 | 4.41 | 15.28 | 1.67 | 8.06 | 20.00 |
| *39* | 17.74 | 17.74 | 33.82 | 33.33 | 40.00 | 27.42 | 20.00 |
| *40* | 33.87 | 20.97 | 55.88 | 37.50 | 48.33 | 53.23 | 70.00 |
| *41* | 0.00 | 0.00 | 16.18 | 8.33 | 13.33 | 12.90 | 20.00 |
| *42* | 20.97 | 16.13 | 19.12 | 26.39 | 41.67 | 40.32 | 10.00 |

**Table 1.** (Contd.)

| Trait no. | AM $n = 62$ | AF $n = 62$ | NM $n = 68$ | NF $n = 72$ | TM $n = 60$ | TF $n = 62$ | CanM $n = 20$ |
|---|---|---|---|---|---|---|---|
| *43* | 0.00 | 0.00 | 0.00 | 2.78 | 8.33 | 9.68 | 15.00 |
| *44*m | 40.32 | 16.13 | 38.24 | 16.67 | 30.00 | 6.45 | 20.00 |
| *45* | 12.90 | 37.10 | 20.59 | 30.56 | 33.33 | 41.94 | 45.00 |
| *46* | 37.10 | 19.35 | 35.29 | 25.00 | 15.00 | 6.45 | 25.00 |
| *47* | 12.90 | 20.97 | 22.06 | 33.33 | 31.67 | 33.87 | 15.00 |
| *48* | 75.81 | 77.42 | 80.88 | 90.28 | 55.00 | 58.06 | 85.00 |
| *49* | 50.00 | 8.06 | 51.47 | 23.61 | 40.00 | 17.74 | 75.00 |
| *50* | 48.39 | 12.90 | 51.47 | 16.67 | 63.33 | 8.06 | 85.00 |

*n* is the number of body sides studied, and for medial (m) traits, *n*/2.

ing (NMDS) procedure of the intergroup phenetic distance (*MMD*) matrix was used. The selection of an appropriate metric for the *MMD* matrix was made based on the highest value of Rohlf's cophenetic correlation coefficient (CCC). The ordination of samples in morphospace based on the matrix of phenetic *MMD* distances in non-metric multidimensional scaling was performed using the Kruskal's minimum stress method (Kruskal, 1964). The reliability of the estimates and the correctness of the number of selected dimensions were checked by the stress value and the regression line in the Sheppard's diagram. The minimum distances between the centroids of the samples in the space of the axes of non-metric scaling were constructed using the MST method, (minimum spanning tree). To assess the hierarchical structure of sample relationships, the UPGMA cluster analysis method (unweighted pairwise association by means) was used.

We also performed a multidimensional assessment of the differences between samples based on individual compositions of manifested and absent phenes in individuals, coded as 1 and 0. Following the recommendations of Astaurov (1974), for bilateral variations, the manifestation of phenes on the left and right sides of an individual's body was considered independently. In this case, medial traits were coded twice as a case of "symmetric" manifestation of the phene on both sides. Based on the obtained matrices of individual manifestations of phene compositions comprising 30 NTTs (a restricted set of traits), we initially performed an ordination of the objects using the Principal component analysis method. Subsequently, using the values of all 30 principal components (PCs), we conducted a Canonical variate analysis of the compared samples (see Vasil'ev, 2005; Ansorge et al., 2009). The choice of a large number of PCs was due to the fact that all traits vary almost independently, and the correlation between them is usually extremely low. Therefore, the shares of variance (%) accounted for by the first and subsequent PCs are relatively small and decrease gradually: 8.14, 5.79, 5.68, 5.32, 5.09, ..., with the last, 30th PC, accounting for 0.59% of the total variance. According to Jolliffe's criterion (Jolliffe, 1986), the critical minimum eigenvalue of 0.1312, which still reflects meaningful information, corresponded to PC 22. However, the cumulative variance at this level explained only 88.6% of the total variance, meaning that a reduction in the number of PCs was not advisable.

The measure of epigenetic variability (*EV*) of each nonmetric trait is the difference from the 50% frequency of occurrence, taken modulo, and for the sample as a whole, it is presented as the average value of all individual traits (Smith, 1981). In accordance with the recommendation of Smith (1981), the *EV* indicator was calculated using the formula

$$EV = 1 - \frac{\sum_{i=1}^{r} |50\% - F_i|}{r50\%},$$

where $r$ is the NTT' number, and $F_i$ is the frequency of occurrence of the $i$-trait. The mean *EV* values in each sample were obtained based on repeated ($n = 10$) bootstrap cycles with random replacement of bilateral nonmetric traits. The series of intermediate sequential *EV* values retained during resampling for each sample were used in their multiple comparisons.

Additionally, to assess the epigenetic variability and group instability of mink development, the *Vm* indicator was used as the volume of the intragroup morphospace formed by the ordinates of group objects (Vasil'ev, 2021). In this case, only samples of caged minks were compared (excluding the small Canadian one), previously randomly aligned by the number of observations (in all cases, $n = 35$). With numerically equal samples, this indicator reflected comparable characteristics of the dispersion of the ordinates of individuals in morphospace. The higher the *Vm* value and the greater the dispersion of the ordinates in morphospace, the less stable the development (Vasil'ev, 2021). Under minimal developmental stress, the *Vm* indicator is lower than under conditions of increased

stress. Calculation of *Vm* as the volume of morphospace inside a convex hull (Cornwell et al., 2006; Vasil'ev, 2021), constructed using the outer edge coordinates of intragroup objects, was calculated using the values of the first three canonical variables (CV1–CV3). For this purpose, the CalculateVolume add-in (by A.G. Kursanov) for Microsoft Office Excel, written based on the convhull function in MatLab, was used. To estimate the standard error of measurement *Vm* ($\pm SE$), the bootstrap technique with random replacement of objects in the sample was also used during resampling ($n = 10$). The conformity assessment of the resampling variables *EV* and *Vm* by the normal distribution law was carried out using the Shapiro–Wilk W-test, and the homogeneity of their sample variances was assessed using Levene's test for homogeneity of variance for mean values. The statistical significance of differences in multiple comparisons of samples for the *EV* and *Vm* indicators was assessed separately for males and females using Welch's F-test. Multiple pairwise comparisons of *EV* and *Vm* were conducted on the basis of the Tukey–Kramer post hoc Q-test. When assessing the influence of the factors "strain" (S), "gender/sex" (G), and their interaction (S × G), the two-way analysis of variance method (two-way ANOVA) was applied to the values of the *EV* and the *Vm* indicators, taking into account the Cohen's $\eta^2$ effect size (Cohen, 1992). Calculations were carried out using the software packages PHEN 3.0 (Vasil'ev, 1995) and PAST 4.12 (Hammer et al., 2001).

## RESULTS

### *Phenetic Distances between Samples Based on a Set of Nonmetric Traits*

The results of comparison of the frequencies of occurrence of phenes for 50 and 30 NTTs (see Tables 1, A4) between the samples of males and females of caged strains of aggressive, non-selected, and tame and male wild American minks are presented in the form of matrices of phenetic distances (*MMD*) taking into account the mean standard deviations (*MSD*) (Table 2). All phenetic distances, *MMD,* calculated for the first expanded set of 50 NTTs, turned out to be statistically significant. The distances calculated for the second restricted set of 30 NTTs are also significant, with the exception of the differences between the samples of males and females of non-selected cage minks ($p > 0.05$).

In the expanded set of traits for 50 NTTs, sex differences were more pronounced than interstrain differences. The greatest phenetic differences were expressed between samples of males and females, and within each sex, the samples of aggressive and tame caged minks were the most distant from each other. It turned out that the *MMD* value between males of the aggressive and tame strains exceeded the distances between them and the sample of wild males of the Canadian population. The average measure of sample uniqueness (*MMU*), which characterizes morphological distinctiveness (morphological disparity), has the lowest values in males and females of non-selected minks, characterizing their central position in the overall morphospace. The greatest distinctiveness was demonstrated in the sample of tame females.

In the restricted set of 30 NTTs, by contrast, interstrain differences were more pronounced than sex differences. When comparing samples of males and females from caged mink strains, the greatest distances were found between aggressive and tame minks. The sample of wild Canadian mink males is phenetically approximately as distant from the males of the caged aggressive and tame minks as they are from each other, but it is closer to the sample of non-selected males. The *MMU* indicator also revealed the lowest values for males and females of the non-selected minks, reflecting the central location of these groups in morphospace. Judging by this indicator, wild Canadian males are somewhat further removed from all the caged male samples. As in the previous case, the sample of tame females deviated most from all the others in the overall morphospace. In this calculation version, the differences between the samples of males and females were significantly reduced. The lowest *MMD* value found between males and females in the non-selected mink strain was statistically insignificant ($p > 0.05$). Differences between the sexes in aggressive minks are also small, but marginally significant. However, residual sex differences between female and male tame minks are somewhat more pronounced and are significant ($p < 0.01$), which indicates an interaction between the factors "strain" and "gender/sex," which was evident in separate comparisons of samples of different sex.

The results of the non-metric multidimensional 3D scaling of both phenetic distance matrices for all three NTT comparison versions are visualized in Fig. 2. In the calculations using the Kruskal's method (Kruskal, 1964), the minimum stress value for the 3D comparison was 0 ("perfect agreement").

Figure 2a shows that, in the expanded comparison, the greatest differences between samples were found along the first non-metric axis (NMDS1) and were related to sex, as all female samples were located in the region of minimal values, while all male samples were located in the region of positive values. The second axis (NMDS2) primarily reflected interstrain differences. The third axis (NMDS3) reflected the characteristics of Canadian wild males to the greatest extent. The dashed minimum spanning tree (MST) superimposed on the graph reflects the short-range connections of Canadian males to the sample of non-selected males.

In the second comparison with a restricted set of traits (Fig. 2b), interstrain differences emerged along the first axis (NMDS1). Representatives of the aggressive and tame mink strains were the most distant from each other, while samples of both sexes from the non-

**Table 2.** Mean measures of divergence (*MMD*) and their standard deviations (*MSD*) calculated for complexes of 50 and 30 nonmetric threshold traits of the skull and mandible between samples of males (M) and females (F) of caged strains of aggressive (A), non-selected (N), and tame (T) American minks and males of the wild population (CanM) from Canada, taking into account the mean measures of their uniqueness (*MMU*)

| Sample: strain, sex | American mink caged strains | | | | | | Wild | *MMU* |
|---|---|---|---|---|---|---|---|---|
| | aggressive | | non-selected | | tame | | | |
| | AM | AF | NM | NF | TM | TF | CanM | |
| Calculation for 50 NTT phenes, including sex-related traits (version 1) | | | | | | | | |
| AM | – | 0.1493 | 0.0462 | 0.1229 | 0.1738 | 0.4318 | 0.1218 | 0.1743 |
| AF | *0.0064* | – | 0.1518 | 0.0390 | 0.2955 | 0.2075 | 0.2453 | 0.1814 |
| NM | *0.0061* | *0.0061* | – | 0.0699 | 0.0828 | 0.2626 | 0.0746 | 0.1146 |
| NF | *0.0060* | *0.0060* | *0.0057* | – | 0.1560 | 0.1288 | 0.1979 | 0.1191 |
| TM | *0.0065* | *0.0065* | *0.0062* | *0.0061* | – | 0.2126 | 0.1629 | 0.1806 |
| TF | *0.0064* | *0.0064* | *0.0061* | *0.0060* | *0.0065* | – | 0.2126 | 0.2689 |
| CanM | *0.0130* | *0.0130* | *0.0127* | *0.0125* | *0.0131* | *0.0130* | – | 0.1956 |
| Calculation for 30 NTT phenes not related to sex (version 2) | | | | | | | | |
| AM | – | 0.0178 | 0.0327 | 0.0541 | 0.1795 | 0.2278 | 0.1238 | 0.1059 |
| AF | *0.0083* | – | 0.0712 | 0.0529 | 0.2529 | 0.2461 | 0.1474 | 0.1314 |
| NM | *0.0079* | *0.0079* | – | 0.0111* | 0.0975 | 0.1297 | 0.0764 | 0.0698 |
| NF | *0.0077* | *0.0077* | *0.0073* | – | 0.0999 | 0.0985 | 0.1255 | 0.0736 |
| TM | *0.0084* | *0.0084* | *0.0080* | *0.0078* | – | 0.0395 | 0.1675 | 0.1394 |
| TF | *0.0083* | *0.0084* | *0.0079* | *0.0077* | *0.0084* | – | 0.2202 | 0.1603 |
| CanM | *0.0167* | *0.0167* | *0.0164* | *0.0162* | *0.0169* | *0.0167* | – | 0.1435 |

Calculation of sex-pooled phenes frequencies of 50 NTT:
for sex-related traits, only frequencies of male samples were used (version 3)

| | A | N | T | CanM | *MMU* |
|---|---|---|---|---|---|
| A | – | 0.0561 | 0.1957 | 0.1290 | 0.1269 |
| N | *0.0045* | – | 0.0802 | 0.0876 | 0.0746 |
| T | *0.0048* | *0.0046* | – | 0.1719 | 0.1492 |
| CanM | *0.0120* | *0.0118* | *0.0121* | – | 0.1295 |

* Statistically insignificant mean measure of divergence (*MMD*); standard deviations (*MSD*) are in italics.

selected strain occupied an intermediate position. The minimum spanning tree (MST), on the one hand, connected the samples of caged non-selected males and wild Canadian males in a common 3D morphospace, while on the other hand, it was connected from the non-selected male strain to the sample of aggressive males. The dendrite extended through the nearest sample of non-selected females to the most distant samples of caged tame mink. All strains had the closest ordinates of males and females, reflecting residual sex differences. The largest intergroup differences between the samples of aggressive and tame minks were found along the first axis (NMDS1), while along the second axis (NMDS2), differences were evident between the samples of caged minks and the sample of males from the wild Canadian population. Small residual differences, primarily related to sex, were found along the third axis (NMDS3). non-selected minks occupied an intermediate position between tame and aggressive minks. Construction of a minimum spanning tree (MST) between the sample centroids revealed that the centroid of wild Canadian mink males in morphospace is located closest to the centroid of non-selected males.

In the third combined version of the NTT set, nonmetric multidimensional scaling revealed a similar overall pattern of intergroup differences (Fig. 2c) as in the second restricted version. Notably, in this case, the greatest interstrain differences were between aggressive and tame minks, and a minimum spanning tree connected the samples of caged non-selected, and Canadian wild minks. It is also evident that tame minks are somewhat more differentiated from the control, non-selected minks than aggressive minks.

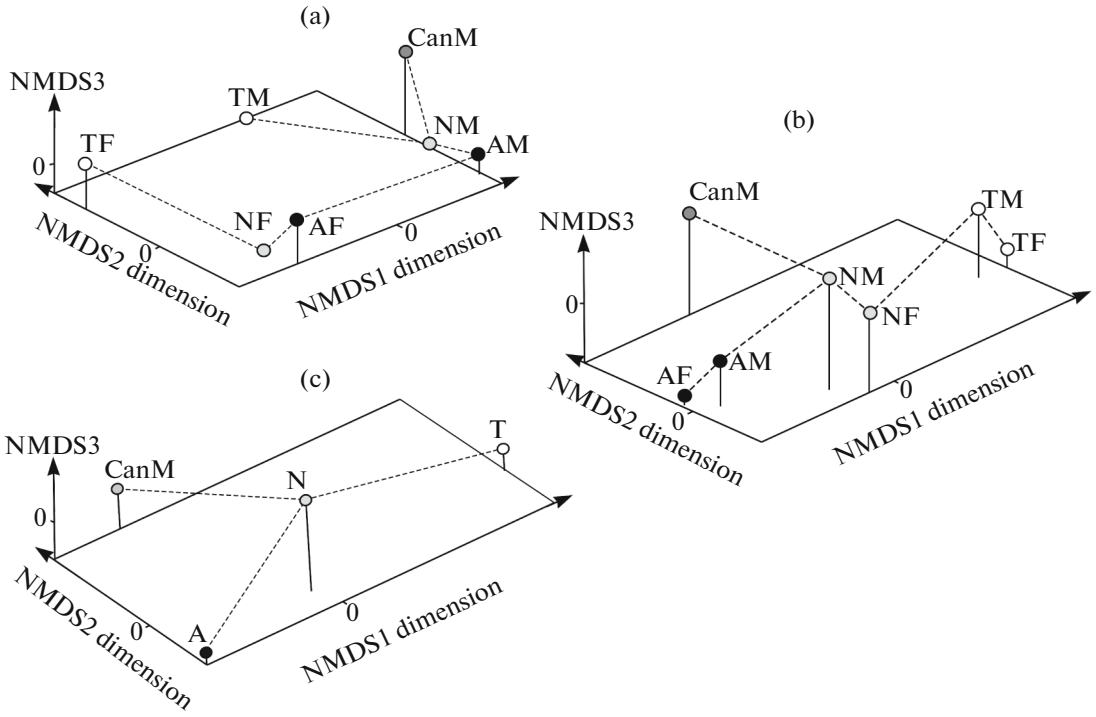


**Fig. 2.** Results of multidimensional non-metric 3D scaling (NMDS) of phenetic distance matrices (mean measures of divergence *MMD*) for three versions of nonmetric trait sets ((a) expanded; (b) restricted; (c) combined) between samples of males (M) and females (F) of aggressive (A), non-selected (N), and tame (T) strains and males of wild (CanM) American minks with the imposition of minimum spanning tree (MST).

The results of the assessment of the hierarchy of relationships between the compared caged and wild mink samples were obtained on the basis of cluster analyses (UPGMA) of three matrices of mean distances *MMD* (Table 2), calculated for 50 and 30 NTTs for three versions of the sets of traits (Figs. 3a–3c). The cluster characterizing the expanded set of traits (3a) clearly revealed two subclusters of males and females. Within the subcluster of males, the branch of tame males (TM) turned out to be the most differentiated, and the sample of Canadian wild males (CanM) occupied an intermediate position between the samples of tame males and other caged aggressive (AM) and non-selected (NM) males. In the second subcluster, among the samples of females, the branch of tame minks (TF) was also the most differentiated.

A different hierarchy of sample relationships emerged from cluster analysis of the phenetic distance matrix *MMD* calculated using a restricted set of NTTs (Fig. 3b). As a result of cluster analysis, with high bootstrap support (100%), the subcluster combining males and females of tame mink strain was significantly differentiated from the other samples. The branch of wild Canadian males, as a basal lineage, joined the subcluster comprising representatives of non-selected and aggressive minks, which combined samples of males and females from their strains in the terminal part of the cluster.

A virtually identical picture of hierarchical relationships was obtained in a cluster analysis of the combined set of traits (Fig. 3c). The cluster structure suggests that tame minks are the most differentiated, while the control sample of wild Canadian individuals occupied an intermediate position between the tame caged strains, on the one hand, and the aggressive and unselected ones, on the other.

### *Canonical Analysis of the Ordinates of the Principal Components of Individual Phenocompositions*

The results of the canonical analysis of the 30 PC values characterizing the manifestation of the ordinates of individual phenocompositions in the compared samples of males and females of caged strains of aggressive, non-selected, tame minks and males of wild individuals of the species from Canada are presented in Fig. 4. The calculation was performed on the basis of the second restricted set of 30 NTTs. Differences along all canonical axes are statistically significant ($p < 0.0001$). The figure shows that, along the first canonical axis, which accounted for 47.32% of the between-group variance, interstrain differences emerged: the samples of aggressive and tame caged minks were the most distant, with non-selected minks occupying an intermediate position. Along the second axis (15.25%), the sample of wild Canadian males was

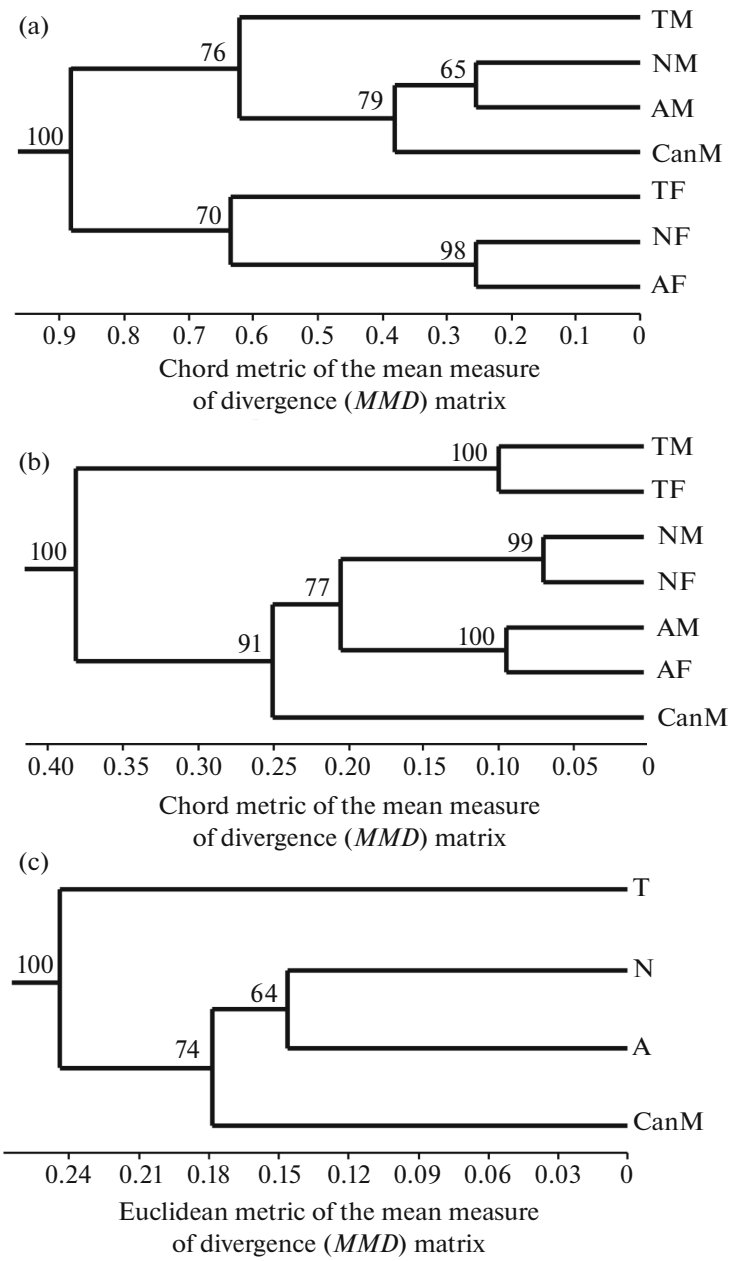


**Fig. 3.** Results of cluster analysis (UPGMA) of phenetic distance matrices (mean measures of divergence, *MMD*) for three versions of sets of nonmetric traits ((a) expanded; (b) restricted; (c) combined) between samples of males (M) and females (F) of aggressive (A), non-selected (N), and tame (T) strais and males of wild (CanM) American minks.

the most distant from all caged minks, with small residual differences between the sexes in all strains. Between-group differences along the third canonical variable (13.61%) were also related to the sex and uniqueness of the wild Canadian individuals. The minimum spanning tree (MST) superimposed on the centroids revealed the greatest proximity of wild Canadian males to the sample of non-selected males.

In contrast to the cumulative retention of some residual sex differences after removing sex-related traits, we did not find significant nonparametric Spearman rank correlation coefficients between the values of the canonical variables characterizing phenetic differences between the three compared mink strains and the condylobasal skull length of individuals in these strains. The values of the Spearman correlation coefficients ($r_S$) ranged from –0.07 to 0.09, and their significance levels ($p$), from 0.346 to 0.472.

### *Evaluation of Epigenetic Variability for a Complex of Nonmetric Trait Phenes*

The results of the evaluation of the indicators of epigenetic variability (*EV*) for a restricted set of NTT

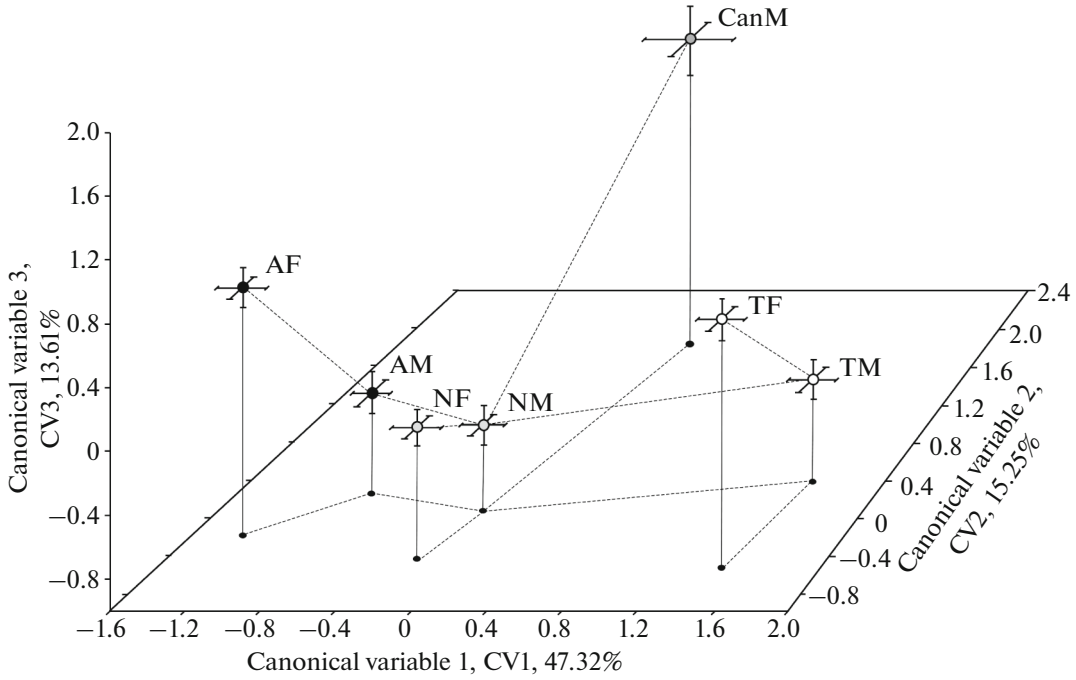


**Fig. 4.** Results of the canonical analysis of the values of the principal components characterizing the individual manifestation of the phenocompositions of 30 nonmetric threshold traits in males (M) and females (F) of aggressive (A), non-selected (N), and tame (T) strains and wild males (CanM) of American minks. The minimum spanning tree (MST) is superimposed on the centroids of the samples (taking into account standard errors ± *SE*).

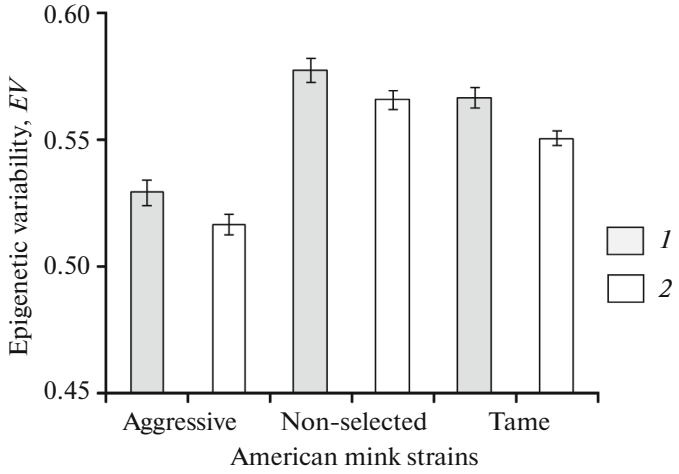


**Fig. 5.** Comparison of the mean index of epigenetic variability (*EV*) with standard errors (±*SE*) for 27 bilateral nonmetric traits of the skull and mandible between samples of males (*1*) and females (*2*) of aggressive, non-selected, and tame strains of American minks.

phenes for the compared samples are presented in Fig. 5. Of the 30 traits, only 27 bilateral traits were included in the comparison. The figure shows that the sex differences in all strains are leveled out, although a weak tendency for slightly higher *EV* values appeared in males. Based on the Shapiro–Wilk W-test, the normal distribution of *EV* for all samples was confirmed (from W = 0.85, $p = 0.060$ to W = 0.95, $p = 0.680$). Using Levene's test for mean values, the homogeneity of sample variances was confirmed (for males $p = 0.855$, for females $p = 0.060$). Multiple comparison of the mean *EV* values between samples of males of the three strains revealed significant differences using the Welch's test: F = 30.02; d.f.= 17.76; $p < 0.0001$, and also between samples of females: F = 43.75; d.f. = 15.21; $p < 0.0001$. In paired comparisons of male samples according to Tukey's post hoc test, the differences were statistically significant between aggressive and

**Table 3.** Results of two-way ANOVAs based on the values of epigenetic variability indices (*EV*) and intragroup morphospace volumes (*Vm*) obtained by resampling between samples of males and females of aggressive, non-selected, and tame American mink strains, taking into account the factors "strain" (S), "gender/sex" (G), and their interaction (S × G)

| Source of variability (factor) | Sum of squares (SS) | Degree of freedom (d.f.) | Mean square (MS) | F | Significance level ($p$) | Effect size ($\eta^2$) | Variance, % |
|---|---|---|---|---|---|---|---|
| Index of epigenetic variability, *EV* | | | | | | | |
| Strain (S) | 0.02532 | 2 | 0.01266 | 79.54 | <0.0001 | 0.75 | 69.00 |
| Gender/sex (G) | 0.00272 | 1 | 0.00272 | 17.08 | 0.0003 | 0.24 | 7.41 |
| S × G | 0.00006 | 2 | 0.00003 | 0.20 | 0.8162 | 0.01 | 0.18 |
| Intragroup | 0.00859 | 54 | 0.00016 | | | | 23.42 |
| Total | 0.03670 | 59 | | | | | 100.00 |
| Index of the intragroup morphospace volume, *Vm* | | | | | | | |
| Strain (S) | 1036.20 | 2 | 518.08 | 168.92 | <0.0001 | 0.86 | 61.19 |
| Gender/sex (G) | 319.23 | 1 | 319.23 | 104.09 | <0.0001 | 0.66 | 18.85 |
| S × G | 172.45 | 2 | 86.22 | 2 8.11 | <0.0001 | 0.51 | 10.18 |
| Intragroup | 165.62 | 54 | 3.07 | | | | 9.78 |
| Total | 1693.50 | 59 | | | | | 100.00 |

tame individuals (Q = 8.12; $p$ = 0.0001), as well as aggressive and non-selected (Q = 10.43; $p$ = 0.0001), but between tame and non-selected ones were not significant (Q = 2.31; $p$ = 0.2483). In a similar comparison of *EV*, all pairwise differences between female samples were significant (from $p$ = 0.0062 to $p$ = 0.0001).

Let us consider the results of the assessment of the ratio of the factors "strain" (S), "gender/sex" (G), and their interaction (S × G) based on a two-way analysis of variance of repeated series of *EV* indicator values for random values obtained by resampling for a complex of 27 bilateral NTT phenes (Table 3). Table 3 shows that the influence of the "strain" and "gender/sex" factors is statistically significant, but their interaction is negligible and insignificant. Accordingly, the share of variance due to the "strain" factor (69.00%) is several times greater than the share of variance associated with sex, which turned out to be significantly smaller (7.41%). The Cohen's effect size for the "strain" factor is significantly higher than the accepted high level ($0.75 > \eta^2 = 0.50$), and for the "gender/sex" factor it only slightly exceeded the average level of differences ($0.24 > \eta^2 = 0.15$). As a result, it was shown that for 27 bilateral nonmetric traits, interstrain differences were expressed mainly according to the indicator of epigenetic variability *EV*.

The results of another analysis of the manifestation of epigenetic variability in the values of the intragroup morphospace volumes, *Vm*, calculated from the values of three canonical variables (CV1–CV3) in randomly aligned caged strain samples according to the number of observations, are presented in Fig. 6. Using the Shapiro–Wilk W-test, the normal distribution of the resampling values of *Vm* for all samples was confirmed (from $p$ = 0.067 to $p$ = 0.577), and using the Levene's test for mean values, we established the homogeneity of sample variances (in males $p$ = 0.204, in females $p$ = 0.130). In multiple comparisons of mean *Vm* values between samples of males of the three strains, the differences assessed using Welch's F-test were significant: F = 39.83; d.f. = 17.24; $p < 0.0001$, as well as between samples of females: F = 103.40; d.f. = 16.94; $p < 0.0001$. In all pairwise comparisons, the differences were also statistically significant (Q criterion estimates according to Tukey's post hoc test ranged from Q = 6.06 to Q = 22.67, and significance levels, from $p$ = 0.0007 to $p$ = 0.0001). The average values of *Vm* were significantly lower in aggressive minks of both sexes than in tame ones (in males: Q = 12.90; $p < 0.0001$; in females: Q = 22.67; $p < 0.0001$). Samples of both sexes of non-selected minks have intermediate values of this indicator: they are significantly higher than those of aggressive males (Q = 6.84; $p$ = 0.0003) and females (Q = 13.68; $p < 0.0001$), but significantly less than in tame males (Q = 6.06; $p$ = 0.0007) and females (Q = 8.99; $p$ = 0.0001). The results of the two-way analysis of variance of the *Vm* values based on their random values obtained during resampling (Table 3) showed that the influence of the factors "strain" (S), "gender/sex" (G), and their interaction (S × G) turned out to be statistically significant ($p < 0.0001$). The share of between-group variance due to the "strain" factor (61.19%) exceeded the shares of variance due to sex (18.85%) and the interaction of factors

(10.18%). Cohen's size effect for both factors and their interaction exceeded the accepted high level (respectively, S, 0.86; G, 0.66; S × G, $0.51 > \eta^2 = 0.50$).

## DISCUSSION

According to Belyaev (1979a), destabilizing selection can increase variability, destabilize historically established morphogenetic patterns, and lead to the reorganization and formation of new adaptive features. Such selection is capable of shaping phenotypic changes not only in the direction of action but also in other directions. These features have largely been observed as a result of selection for defensive behavior characters. Under destabilizing selection, the spontaneous emergence of new, atypical fur color morphs was observed in American minks (Trapezov, 1987), and morphometric changes in the skull and mandible were also detected in aggressive and tame minks (Kharlamova et al., 2000). In this regard, the use of nonmetric trait phenes for indirectly assessing probable epigenetic changes that occurred after selection of American mink strains for defensive behavior characters is entirely justified as another approach to studying the problem of domestication.

In the case of the expanded 50 NTT set, it is not surprising that the main phenetic differences, including phenetic distance values (*MMD*), non-metric scaling results, and cluster analysis results, revealed clear sex-related differences between the compared samples, as this set included traits significantly correlated with sex and size. This comparison revealed that the magnitude of sex-related differences exceeded interstrain differences and was generally greater than the differences between control samples of non-selected caged and wild Canadian males. Another important point is that interstrain differences in males and females manifested themselves in parallel: the samples from aggressive and tame caged mink strains were the most distant from each other, with the non-selected control samples occupying an intermediate position.

In the restricted 30 NTT set, all the phenes used are not related to the size and sex of the animals, have frequencies that vary between samples, and are also not related in their manifestation to each other, i.e., intergroup differences are largely due to hereditary rather than environmental factors, which allows for a genetic and epigenetic interpretation of the differences.

Since sexual dimorphism is typical for most species of mustelids (Abramov and Tumanov, 2003; Loy et al., 2004; Gálvez-López et al., 2021), we had to exclude NTTs that were significantly sex-related in their expression in order to isolate the actual interstrain differences in their occurrence. After removing a series of traits the expression of which was significantly sex-related, some traits still retained some residual weakly sex-related variability, cumulatively leading to a small divergence in the centroids of males and females in the morphospace (see Fig. 3). Residual sex-related phenetic differences are generally small: they are insignificant between males and females of non-selected minks ($p > 0.05$), while in the aggressive strain, sex differences only slightly exceeded the first level of significance ($p < 0.05$) and only in the tame strain were sex differences clearly expressed ($p < 0.01$). In this comparison, sex differences are significantly smaller than interstrain differences. In this case, the goal was not to combine samples of different sexes, but to reduce the influence of environmental factors by excluding NTTs associated with sexual dimorphism in body size. Moreover, the unequal degree of sex differences in different strains reflects the interaction of the "strain" and "gender/sex" factors, which is important to consider and requires a sex-separated analysis of NTTs.

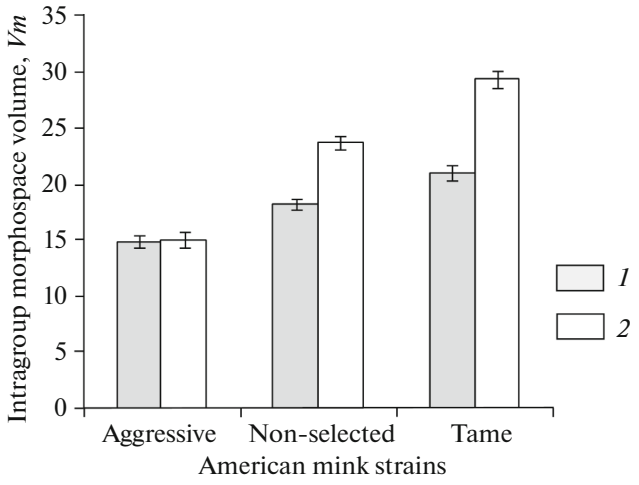


**Fig. 6.** Comparison of the mean volumes of intragroup morphospaces *Vm* of samples of (*1*) male and (*2*) female American minks (taking into account standard errors ± *SE* based on resampling), calculated from the values of the first three canonical variables (CV1–CV3).

In the third version of calculations using the combined set of 50 NTTs, the ratio of intergroup differences was virtually identical to that obtained in the second version of calculations using the restricted set of traits. Therefore, the enhancement of interstrain heritable differences due to the sharp reduction in the contribution of sex-related traits demonstrated the validity of this approach. Previously, other authors also conducted a similar comparison of male and female samples in mustelids after excluding some sex-related traits (Ranyuk and Monakhov, 2011).

A comparison of phene frequencies across all three NTT sets revealed that the degree of differentiation between one of the wild Canadian populations and caged strains that are distant descendants of wild mink bred for many generations in captivity on fur farms over the course of a century was less pronounced than between aggressive and tame caged strains obtained through selective breeding. Moreover, the duration of

selection on the experimental fur farm was significantly shorter than the time mink have existed on fur farms. In all comparison sets, wild Canadian mink were closest in phene frequencies to non-selected caged strains. The final cluster analysis for the combined NTT set also confirmed that wild Canadian mink were less differentiated from caged strains of captive mink than the caged aggressive and tame mink were between themselves. The identified differences in the NTT complex between caged minks and wild Canadian minks are in good agreement with the results of Korablyov et al. (2018) for wild and farmed minks from a fur farm in Tver oblast. In both cases, farmed and wild minks differed in NTT frequencies, potentially allowing them to be distinguished. It is known that the skulls of American minks obtained from fur farms are larger than those of wild individuals (Lynch and Hayden, 1995; Sidorovich et al., 1999). The possibility of distinguishing between the skulls of "domesticated" (escapees from fur farms) and wild American minks based on morphometry has been demonstrated (Tamlin et al., 2009). All this indicates a high phenotypic plasticity of the species in both size and structural morphological traits, as well as its high adaptive and selection potential.

As a result of selection for defensive behavior features over a relatively small number of generations, both strains of aggressive and tame minks significantly deviated from the "original" NTT phene frequencies, such that they diverged from them in opposite directions in the morphospace. A similar picture of hierarchical relationships between the compared caged and wild mink samples was revealed both by the phenetic distance matrix (see Table 2) and its visualization with non-metric multidimensional scaling (see Fig. 3), and by canonical analysis (see Fig. 4) of individual phenocompositions. The results of the different analysis methods are in good agreement, and the minimum spanning trees (MST) reflect the same structure of connections between the same samples. The samples of males and females from the tame mink strain were the most distant in the morphospace from the other samples in all comparison variants. A high degree of NTT frequency variation was detected particularly in tame female minks. For a number of NTTs, the tame mink strain exhibited sharp changes in phene frequencies (e.g., for the traits: *1,* FiCrns(–); *42*, FeFocnif (FFCI); *43,* FJginap), which can be interpreted as the manifestation of new properties compared to other caged and wild minks.

The results of assessing the average indcators of epigenetic variability (*EV*) and the volumes of intragroup morphospace (*Vm*) indicate an increase in epigenetic variability, developmental instability, and the degree of morphological disparity in representatives of tame minks compared to aggressive ones. All these features of the experimental strain of tame minks are in good agreement with Belyaev's theoretical concepts on the role of destabilizing selection (Belyaev, 1979a; Belyaev and Trut, 1989). A decrease in variability in the strain of aggressive minks as a result of selection for behavioral features may indirectly indicate the emergence of structural constraints on morphogenesis. The emergence of such a limitation on variability can also be considered a special form of developmental destabilization.

It can be assumed that the factor of regular stress of animals on a fur farm in contact with humans could contribute to the activation of epigenetic rearrangements associated with morphogenetic changes that can be transgenerationally preserved in generations of mink strains and fixed in their genomes. Similar transgenerational effects have been shown in a series of studies on other objects (Jablonka and Raz, 2009; Skinner et al., 2014; Burggren, 2016; Bošković and Rando, 2018; Donelan et al., 2020). Earlier, Badyaev et al. (2005) found that, following deforestation and burning of forest debris, shrew species experienced severe stress in the altered biotopes, which increased their embryonic mortality, reduced their abundance, and caused an increase in epigenetic variability, assessed by the authors based on the manifestations of morphological anomalies and developmental instability. We agree with Badyaev (2014) that morphogenetic changes may be due to stress-induced epigenetic changes. Donelan et al. (2020) examined the phenomenon of environmental stress-induced transgenerational phenotypic plasticity, which arises through rapid epigenetic rearrangements. Its leading role is manifested in the preservation and transmission of new beneficial adaptive modifications across generations. It can be assumed that transgenerational inheritance of emerging epigenetic changes affecting morphogenesis can preserve newly established epigenetic threshold mechanisms that determine the frequency of NTT phenes in American mink strains. Further molecular genetic analysis of the epigenetic DNA profiles of American mink strains may help clarify these issues.

## CONCLUSIONS

Research has indirectly demonstrated that selection for defensive behavior characters in mink has led to differences in the epigenetic system of experimental strains of aggressive and tame American mink, related to the morphogenesis of certain NTTs. The frequencies of nonmetric trait phenes in the aggressive and tame strains changed significantly compared to non-selected strains, many of them in opposite directions. At the same time, judging by the phenetic distance (*MMD*), the non-selected mink strain largely retained the characteristic frequencies of nonmetric traits that were expressed in wild Canadian individuals. The latter can be tentatively classified as the source population for modern caged mink, which have been maintained on fur farms for over a century. The interstrain differences in NTT phene frequencies achieved

through selection for defensive behavior features turned out to be quite large, despite the relatively small number of generations of destabilizing selection.

The relatively rapid changes in NTT phene frequencies we observed during the divergence of experimental strains of aggressive and tame minks indirectly indicate the high rate of epigenetic rearrangements in the two experimental strains of American mink. These findings can be considered as the result of selection fixing of the initially available reserve modification variants of the species through probable stress-induced epigenetic changes associated with the determination of specific morphogenetic pathways.

The divergence of the strains in the NTT complex indirectly indicates that a number of nonmetric cranio-mandibular traits were also subject to selective changes. Therefore, contrary to the idea that NTTs are subject to only weak selective pressure, they may, on the contrary, experience strong selective pressure due to their association with other functionally important characteristics. Therefore, NTTs, as structural morphological traits, can serve as good markers of selectively induced morphogenetic rearrangements, indirectly reflecting the degree of genetic and epigenetic changes. The obtained results not only indicate a relatively high rate of epigenetic rearrangements in the experimental strains of American mink but also may indirectly explain the high adaptive potential of an invasive species—the American mink—during the rapid expansion of its new range in Eurasia.

## *APPENDIX*

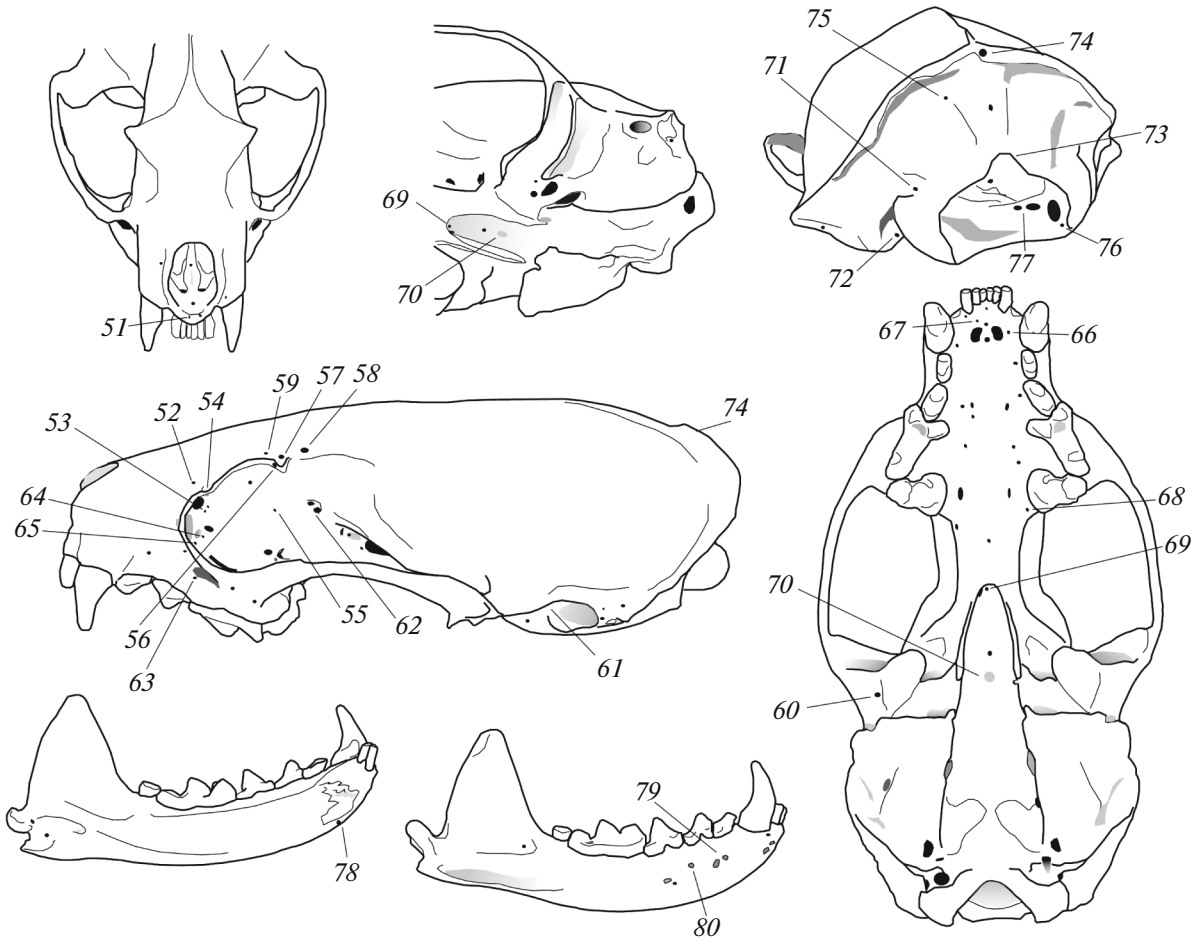


**Fig. A7.** The arrangement of detected but not-used nonmetric threshold traits on the skull and mandible of the American mink (nos. *51–80*): *51**m, FPmdsI1; *52* (n), FFrlc; *53* (n), FPlcve; *54* (n), TbLcrd; *55**, FFrob; *56**, FSpb; *57**, PrSobpf; *58**, FSobpo; *59* (n), FSoban; *60* (n), FTmve; *61* (n), CnAumd(–); *62**, FEtdu; *63* (n), FIoanac; *64* (n), CnIoacit; *65* (n), CnIoacex; *66* (n), FIcla; *67**, FIcan; *68**, FAvM1; *69**, FPspve; *70* m*, FeSph; *71* (n), FCdlads; *72* (n), FCdlave; *73*m(n), FMgtg; *74**, FPtoc; *75* (n), FOclads; *76**, FCditac; *77**, FHgitdu; *78**, FMdsm; *79**, FMtdu; *80**, FMtim (* numbers of traits with invariant frequencies in the samples; m, medial trait; (n) numbers of nonvalid traits; full names and descriptions of the traits are provided in Table A4, and the reasons for their rejection are given in Table A5.

**Table A4.** List of studied phenes of nonmetric threshold traits (NTTs) of the axial skull and mandible of the American mink

| No. | Description of the phene of nonmetric trait | Code |
|---|---|---|
| *1* | Absence of the medial fissure in the nasal crest of the palatine process of the maxilla, Fissura medialis in crista nasalis (–) | FiCrns (–) |
| *2* | The opening in the nasal ridge behind the cleft, Foramen posterior in crista nasalis | FCrnspo |
| *3* | Median nasal opening on the premaxillary bone, Foramen premaxillare nasale mediale | FPmnsme |
| *4* | Dorsal opening of the premaxillary bone above the second incisor, Foramen premaxillare dorsale Super I2 | FPmdsI2 |
| *5* | The superior opening on the nasal process of the premaxillary bone (on the sides of the pyriform opening), Foramen premaxillare nasale dorsale | FPmnsds |
| *6* | Inferior opening on the nasal process of the premaxillary bone (on the sides of the pyriform opening), Foramen premaxillare nasale ventrale | FPmnsve |
| *7* | The opening in the vestibule of the lacrimal canal, in its middle part, Foramen paralacrimale intermedium | FPlcim |
| *8* | Anterior frontal opening (to the supraorbital process), Foramen frontale anterius | FFran |
| *9* | Absence of the temporal foramen, Foramen temporale (–) | FTm (–) |
| *10* | Anterior opening on the zygomatic process of the maxilla, Foramen maxillaris zygomaticus anterius | FMxzgan |
| *11* | Posterior opening on the zygomatic process of the maxilla, Foramen maxillaris zygomaticus posterius | FMxzgpo |
| *12* | The post-articular opening of the squamous bone in front of the auditory canal ("temporal meatus"), Foramen postglenoidale | FPgl |
| *13* | Lateral opening in the posterior external wall of the auditory canal, Foramen accessorium laterale in meatus acusticus | FMtacla |
| *14* | Dorsal opening in the outer wall of the auditory canal, Foramen accessorium dorsale in meatus acusticus | FMtacds |
| *15* | Lateral opening in the squama of the temporal bone above the occipital crest behind the auditory opening, Foramen squamosum laterale | FSqla |
| *16* | Maxillary foramen in the canine fossa, Foramen maxillare in fossa canina | FMxca |
| *17* | Absence of an opening in the maxilla in the area of the orbit inside the preorbital opening, Foramen maxillare orbitale (–) | FMxor (–) |
| *18* | The opening on the upper jaw in the eye socket area is the Fenestra maxillaris orbitalis | FeMxor |
| *19* | Additional opening within the preorbital foramen (anterior to the ventral orbital foramen), Foramen infraorbitale accessorium | FIoac |
| *20* | Accessory opening to the ventral sphenopalatine foramen, Foramen sphenopalatinum accessorium | FSplac |
| *21* | Additional opening inside the optic canal, Foramen opticum accessorium | FOpac |
| *22* | Fossa anterior to the optic canal, Fossa praeopticus | Fspop |
| *23* | Foramen in fissura orbitalis (on the ventral part) | FFior |
| *24* | Presence of a round opening, Foramen rotundum (+) | FRt (+) |
| *25* | Additional dorsal opening above the round one, Foramen rotundum accessorium | FRtac |
| *26* | Additional middle interincisor foramen, Foramen incissivum media accessorium | FIcmeac |
| *27* | Lingual alveolar foramen of the third upper incisor, Foramen alveolare linguale I 3 | FAvlgIn |
| *28* | Lingual alveolar foramen of the canine, Foramen alveolare linguale Canini | FAvlgC |
| *29* | Lingual foramen at the alveolus of the first premolar, Foramen alveolare linguale Pm1 | FAvlgPm1 |
| *30* | Lingual foramen at the alveolus of the second premolar, Foramen alveolare linguale Pm2 | FAvlgPm2 |
| *31* | Multiple (more than two) anterior palatine (maxillary) openings, Foramina maxillaris palatina multiplex | FMxplmt |
| *32* | Additional palatine opening in the upper carnassial tooth sector, Foramen palatinum Pm3 accessorium | FPlPm3ac |
| *33* | Postmolar hole, Foramen molaris posterius | FMpo |

**Table A4.** (Contd.)

| No. | Description of the phene of nonmetric trait | Code |
|---|---|---|
| *34* | Medial palatine foramen, Foramen palatinum medium | FPlme |
| *35* | Lateral notch of the presphenoid bone of the presphenoid bone, Incissura praesphenoidalis lateralis | IsPspla |
| *36* | The medial opening between the pterygoid processes of the sphenoid bone, Foramen sphenoideum medium | FSpme |
| *37* | Incissura lamina pterygoidei | IsLmpt |
| *38* | Openings on the tympanic chamber, Fenestra tympanica | FeTp |
| *39* | Accessory opening of the facial canal, Foramen stylomastoideum accessorium | FStmac |
| *40* | Anterior tympanic process of the temporal bone in the shape of a "papilla" in contact with the basilar part of the sphenoid bone, Processus tympanicus anterior | PrTpan |
| *41* | An opening in the occipito-tympanic suture behind the carotid foramen, Foramen occipito-tympanicum | FOctp |
| *42* | A fenestra in the inferior condylar fossa on the ventral side, Fenestra in fossae inferioris condylus occipitalis (FFCI according to Monakhov, 2010) | FeFocnif (FFCI) |
| *43* | The internal jugular foramen opens into the inferior condylar fossa, Foramen jugulare internum apertum in fossa condylaris inferior | FJginap |
| *44* | Medial opening in the squama of the occipital bone, Foramen supraoccipitale medium | FOcme |
| *45* | Double incisive mental foramen, Foramen mentale incissivum duplicatum | Fmticdu |
| *46* | An additional opening on the ventral surface of the mandible at the alveolus of the lower canine tooth, Foramen mandibulare alveolare caninum ventrale | FMdcave |
| *47* | Double posterior mental foramen, Foramen mentale posterius duplex | Fmtpodu |
| *48* | Foramen on the masseteric fossa of the mandible, Foramen mandibulare massetericus | FMdms |
| *49* | Foramen on the lingual side of the angular process of the mandible, Foramen mandibulare angulare linguale | FMdaglg |
| *50* | Dorsal foramen of the incisive bone above the first incisor, Foramen mandibulare articulare linguale | FMdarlg |
| *51* | Dorsal opening of the intermaxillary bone above the first incisor, Foramen intermaxillare dorsale super I1 | FPmdsI1 |
| *52* | The opening on the frontal bone in front of the lacrimal tubercle (anterior lacrimal), Foramen frontale lacrimale | FFrlc |
| *53* | The opening in the vestibule of the lacrimal canal in the lower part (ventral), Foramen praelacrimale ventrale | FPlcve |
| *54* | Reduction of the lacrimal process (tubercle),Tuberculum lacrimalis reductus | TbLcrd |
| *55* | Orbital frontal opening, Foramen frontale orbitale | FFrob |
| *56* | Foramen in the supraorbital process, Foramen supraorbitale | FSpb |
| *57* | Through hole in the supraorbital process, Processus supraorbitalis perforatus | PrSobpf |
| *58* | The opening of the frontal bone behind the supraorbital process, Foramen frontale posterius | FSobpo |
| *59* | The opening of the frontal bone behind the supraorbital process, Foramen frontale anterius | FSoban |
| *60* | Ventral temporal opening, Foramen temporale ventrale | FTmve |
| *61* | Absence of a medial canal within the auditory canal, Canalis medialis in meatus acusticus (–) | CaAcmd (–) |
| *62* | Doubled ethmoidal opening, Foramen ethmoideum duplicatum | FEtdu |
| *63* | Anterior accessory maxillary foramen within the preorbital above Pm2, Foramen infraorbitale accessorium | FIoanac |
| *64* | Internal accessory opening to the infraorbital canal, Canalis infraobitalis accessorius internus | CnIoacit |
| *65* | External accessory opening to the infraorbital canal, Canalis infraobitalis accessorius externus | CnIoacex |
| *66* | Additional lateral incisive foramen, Foramen incissivum laterale | FIcla |
| *67* | Additional anterior interincisor foramen, Foramen incissivum anterius | FIcan |
| *68* | Alveolar foramen of the first upper molar, Foramen alveolare M1 | FAvM1 |

**Table A4.** (Contd.)

| No. | Description of the phene of nonmetric trait | Code |
|---|---|---|
| *69* | Foramen of the presphenoid bone beneath the palate, Foramen praesphenoidale ventrale | FPspve |
| *70* | Medial opening between the pterygoid processes of the sphenoid bone, Fenestra sphenoidalis. | FeSph |
| *71* | Dorsal lateral opening in the superior condylar fossa, Foramen condyare laterale dorsale | FCdlads |
| *72* | Ventral lateral condylar foramen, Foramen condylare laterale ventral | FCdlave |
| *73* | Triangular shape of the foramen magnum, Foramen magnum habet figures triangular | FMgtg |
| *74* | Bilateral opening in the occipital pretubercles, Foramen in protuberantia occipitalis | FPtoc |
| *75* | Lateral dorsal opening in the squama occipitalis, Foramen dorsale laterale in squama os occipitalis | FOclads |
| *76* | Additional foramen to the internal condylar (ventral-lateral), Foramen condyloideum internum accessorium | FCditac |
| *77* | Double internal hypoglossal foramen, Foramen hypoglossum internum duplex | FHgitdu |
| *78* | Symphysial opening of the mandible, Foramen mandibulare symphysiale | FMdsm |
| *79* | The main (anterior) mental foramen is doubled, Foramen mentale duplex | FMtdu |
| *80* | Presence of the intermediate mental foramen, Foramen mentale intermedium | FMtim |

ac, accessorius (-um); an, anteror (-ius); ap, apertus (-a, -um); ds, dorsalis (-e); du, duplexs; ex, externus (-a, um); im, intermedius (-um); in, internus (-a, um); la, lateralis (-e); lg, lingualis (-e); me, medialis (-e); mt, multiplex; ns, nasalis (-e); or, orbitalis (-e); pf, perforates; po, posteror (-ius); rd, reductus; tr, triangularis; ve, ventralis (-e).

**Table A5.** List of valid and excluded nonmetric traits of the axial skull and mandible of the American mink and the reasons for their rejection

| No. | Trait code | Trait validity | Significant correlation | | Invariant frequencies ($\chi^2 < 12.6$, $p > 0.05$) |
|---|---|---|---|---|---|
| | | | with size | with sex | |
| *1* | FiCrns (–) | 1, 2, 3 | | | |
| *2* | FCrnspo | 1, 2, 3 | | | |
| *3* | FPmnsme | 1, 3 | * | * | |
| *4* | FPmdsI2 | 1, 2, 3 | | | |
| *5* | FPmnsds | 1, 3 | * | * | |
| *6* | FPmnsve | 1, 3 | * | * | |
| *7* | FPlcim | 1, 2, 3 | | | |
| *8* | FFran | 1, 3 | * | * | |
| *9* | FTm (–) | 1, 2, 3 | | | |
| *10* | FMxzgan | 1, 3 | * | * | |
| *11* | FMxzgpo | 1, 3 | * | * | |
| *12* | FPgl | 1, 2, 3 | | | |
| *13* | FMtacla | 1, 3 | * | * | |
| *14* | FMtacds | 1, 3 | * | * | |
| *15* | FSqla | 1, 2, 3 | | | |
| *16* | FMxca | 1, 2, 3 | | | |
| *17* | FMxor (–) | 1, 2, 3 | | | |
| *18* | FeMxor | 1, 2, 3 | | | |
| *19* | FIoac | 1, 2, 3 | | | |
| *20* | FSplac | 1, 3 | * | | |
| *21* | FOpac | 1, 3 | * | * | |
| *22* | Fspop | 1, 3 | * | * | |
| *23* | FFior | 1, 2, 3 | | | |
| *24* | FRt (+) | 1, 2, 3 | | | |
| *25* | FRtac | 1, 2, 3 | | | |

**Table A5** (Contd.)

| No. | Trait code | Trait validity | Significant correlation | | Invariant frequencies ($\chi^2 < 12.6$, $p > 0.05$) |
|---|---|---|---|---|---|
| | | | with size | with sex | |
| *26* | FIcmeac | 1, 2, 3 | | | |
| *27* | FAvlgIn | 1, 3 | * | * | |
| *28* | FAvlgC | 1, 3 | * | * | |
| *29* | FAvlgPm1 | 1, 3 | * | * | |
| *30* | FAvlgPm2 | 1, 2, 3 | | | |
| *31* | FMxplmt | 1, 2, 3 | | | |
| *32* | FPlPm3ac | 1, 2, 3 | | | |
| *33* | FMpo | 1, 2, 3 | | | |
| *34* | FPlme | 1, 3 | * | * | |
| *35* | IsPspla | 1, 2, 3 | | | |
| *36* | FSpme | 1, 3 | * | | |
| *37* | IsLmpt | 1, 3 | * | * | |
| *38* | FeTp | 1, 2, 3 | | | |
| *39* | FStmac | 1, 2, 3 | | | |
| *40* | PrTpan | 1, 2, 3 | | | |
| *41* | FOctp | 1, 2, 3 | | | |
| *42* | FeFocnif (FFCI) | 1, 2, 3 | | | |
| *43* | FJginap | 1, 2, 3 | | | |
| *44* | FOcme | 1, 3 | * | * | |
| *45* | Fmticdu | 1, 2, 3 | | | |
| *46* | FMdcave | 1, 2, 3 | | | |
| *47* | Fmtpodu | 1, 2, 3 | | | |
| *48* | FMdms | 1, 2, 3 | | | |
| *49* | FMdaglg | 1, 3 | * | * | |
| *50* | FMdarlg | 1, 3 | * | * | |
| *51* | FPmdsI1 | 0 | | | * |
| *52* | FFrlc | n | * | * | |
| *53* | FPlcve | n | | * | |
| *54* | TbLcrd | n | * | * | |
| *55* | FFrob | 0 | | | * |
| *56* | FSpb | 0 | * | | * |
| *57* | PrSobpf | 0 | * | * | * |
| *58* | FSobpo | 0 | | | * |
| *59* | FSoban | n | * | * | |
| *60* | FTmve | n | * | * | |
| *61* | CaAcmd (–) | n | * | * | |
| *62* | FEtdu | 0 | | | * |
| *63* | FIoanac | n | * | * | |
| *64* | CnIoacit | n | * | * | |
| *65* | CnIoacex | n | | * | |
| *66* | FIcla | n | * | * | |
| *67* | FIcan | 0 | | | * |
| *68* | FAvM1 | 0 | | * | * |

**Table A5** (Contd.)

| No. | Trait code | Trait validity | Significant correlation | | Invariant frequencies ($\chi^2 < 12.6$, $p > 0.05$) |
|---|---|---|---|---|---|
| | | | with size | with sex | |
| *69* | FPspve | 0 | * | | * |
| *70* | FeSph | 0 | * | | * |
| *71* | FCdlads | n | | * | |
| *72* | FCdlave | n | * | * | |
| *73* | FMgtg | n | | * | |
| *74* | FPtoc | 0 | * | | * |
| *75* | FOclads | n | * | * | |
| *76* | FCditac | 0 | | * | * |
| *77* | FHgitdu | 0 | * | | * |
| *78* | FMdsm | 0 | | * | * |
| *79* | FMtdu | 0 | * | | * |
| *80* | FMtim | 0 | * | | * |

1, 2, 3, valid traits: 1, an expanded set of 50 NTTs, partially related to sex and size; 2, a restricted set of 30 NTTs, not related to sex and size; 3, a combined set of 50 NTTs, where only the frequencies of male traits are included for sex-related traits. Excluded traits: 0, with invariant frequencies in samples; *n*, invalid, with fuzzy topology, leading to significant shifts in frequency of occurrence during reclassification.

## SUPPLEMENTARY INFORMATION

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research was carried out on museum collection material, originally obtained at an experimental fur farm at the Federal Research Center of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, in compliance with the rules, ethical standards, and protocols for conducting scientific research using experimental animals, approved by the order of the Presidium of the USSR Academy of Sciences dated April 2, 1980, no. 12000-496 and the order of the USSR Ministry of Higher Education dated September 13, 1984, no. 2, and in accordance with Protocol no. 13 of the meeting of the Bioethics Commission of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (Yekaterinburg), dated November 1, 2022.

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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