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The three-dimensional shape changes of the American mink (Carnivora, *Neogale*) skull under aggressive and tame behaviour selection

Aleksey G. Vasil'ev*, Irina A. Vasil'eva, Mikhail V. Chibiryak & Oleg V. Trapezov

ABSTRACT. Domestication morphogenetic effects resulting from long-term (16–17 generations) selection based on characters of defensive behavior in the American mink, *Neogale vison* (Schreber, 1777), were studied. A comparative study of the size and shape variations of the skull among obtained experimental mink strains (aggressive and tame) using 3D geometric morphometrics was carried out. The control was a non-selected minks kept in parallel. Samples of wild American and European minks (*Mustela lutreola* L., 1759) were used to assess the extent of the changes that occurred. In the presence of pronounced sexual dimorphism in size in all groups, a significant decrease in the skull size of tame minks was revealed. The greatest differences in the skull shape were found between aggressive and tame minks. They are comparable to the differences between cage and wild American minks, exceeding half of the interspecific differences. Tame minks exhibit skull changes in accordance with the domestication syndrome: relative shortening of the facial part, an increase in height in the frontal bone area, narrowing of the between-orbit interspace, reduction of canines. The level of morphogenetic rearrangements of the skull shape between aggressive and tame minks as a result of selection is quite high and comparable to microevolutionary transformations. Selection based on behavioral traits led both tame and aggressive minks to destabilization of skull development, which is consistent with the theory of destabilizing selection by Dmitri Belyaev.

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Трехмерные изменения формы черепа американской норки (Carnivora, *Neogale*) в результате селекции на агрессивное и ручное поведение

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РЕЗЮМЕ. Изучены морфогенетические эффекты доместикации, возникающие в результате длительного (16–17 поколений) отбора, основанного на признаках оборонительного поведения американской норки, *Neogale vison* (Schreber, 1777). Проведено сравнительное исследование вариаций размеров и формы черепа у полученных экспериментальных линий норок (агрессивных и ручных) с использованием 3D геометрической морфометрии. В качестве контроля использовались неселектированные норки, содержавшиеся параллельно. Для оценки степени произошедших изменений использованы выборки диких американских и европейских норок (*Mustela lutreola* L., 1759). При наличии во всех группах выраженного полового диморфизма в размерах было выявлено значительное уменьшение размеров черепа у ручных норок. Наибольшие различия в форме черепа были обнаружены между агрессивными и ручными норками. Они сопоставимы с различиями между клеточны-

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ми и дикими американскими норками, превышая половину межвидовых различий. У ручных норок наблюдаются изменения черепа в соответствии с доместикационным синдромом: относительное укорочение лицевой части, увеличение высоты лобной кости, сужение межглазничного пространства, уменьшение клыков. Уровень морфогенетических перестроек формы черепа между агрессивными и ручными норками в результате селекции достаточно высок и сопоставим с микроэволюционными преобразованиями. Отбор, основанный на поведенческих признаках, привел как ручных, так и агрессивных норок к дестабилизации развития черепа, что согласуется с теорией дестабилизирующего отбора Д.К. Беляева.

КЛЮЧЕВЫЕ СЛОВА: селекция, оборонительное поведение, *Neogale vison*, изменчивость черепа, постнатальный морфогенез, геометрическая морфометрия.

Introduction

Domestication and related morphological changes have attracted the attention of researchers for many years as a model of rapid evolutionary phenomena (Darwin, 1868; Belyaev, 1979; Belyaev & Trut, 1989; Trapezov, 2012; Kaiser *et al*., 2015; Lord *et al*., 2020). Representatives of Belyaev's scientific school established the influence of experimental selection based on characters of defensive behavior on the morphological variation in a number of species of carnivores and rodents (Belyaev & Trut, 1989; Kharlamova *et al*., 2000; Plyusnina *et al*., 2011; Trapezov, 2012). As a result of the selection the appearance of some specific traits is well known, among them there are piebaldness in fur coloration, changes in the shape of the tail and ears, animal vocalization, their hormonal background, as well as the size and shape of the skull (Trut *et al*., 1991; Kharlamova *et al*., 2000; Singh *et al*., 2017). It is assumed that these hormonal and morphogenetic changes are associated with the initial period of formation of neural crest cells (NCC), when a domestication syndrome may develop with an initial deficit of NCC (Wilkins *et al*., 2014; Wilkins, 2017; Lord *et al*., 2020). As a result, the development of a number of tissues is recursively affected, including the formation of pigment cells, as well as the adrenal gland, teeth and bones of the facial part of the skull. Thus, when studying rat strains (*Rattus norvegicus* (Berkenhout, 1769)), which were selected for aggressive and tame behavior (Singh *et al*., 2017), the manifestation of typical changes in the shape of the facial part of the skull was revealed. In particular, the strain of tame individuals demonstrated the increased skull height at the level of the frontal bone (pug-likeness) and some violations in the teeth structure. Similar characters are also typical for a number of dog breeds (Drake & Klingenberg, 2010) and experimental strains of silver-black foxes (Trut *et al*., 2021).

The American mink, *Neogale vison* (Schreber, 1777), was similarly subjected to prolonged experimental selection based on characters of defensive behavior towards humans, aimed at breeding aggressive and tame strains on an experimental fur farm of the Institute of Cytology and Genetics of the Siberian Branch of RAS (Trapezov, 1987, 2012). At an early stage of selection (9–11 generations), it was possible to detect changes in some skull measurements in both the aggressive and tame mink strains (Kharlamova *et al*., 2000). Now since the initial craniometric comparison, about 10 generations of breeding of experimental strains of aggressive and tame American minks have passed based on characters of defensive behavior, which could be accompanied by an increase in differentiation according to cranial traits. On the other hand, in this species, like many other mustelids, intergrowth and dense fusion of many skull bones occurs at an early age, which probably has a biomechanical functional significance, but at the same time it can potentially limit the skull variability. Compared with relatively labile cranial rearrangements in canids and rodents (Trut *et al*., 1991; Drake & Klingenberg, 2010; Lord *et al*., 2020) this circumstance hypothetically can reduce the potential morphogenetic skull changes in the American mink during domestication and requires its own study.

In addition, when comparing the aggressive and tame strains of American minks (Kharlamova *et al*., 2000), the relative scale of cranial morphometric changes remained unexplored. It is surely worth to compare the measure of interstrain differentiation with the levels of their morphological divergence from wild Canadian population of American mink and the other species of mustelids — the European mink, *Mustela lutreola* Linnaeus, 1758 (Carnivora: Mustelidae). In this regard, it becomes necessary to revise the cranial differences between the strains that arose at the next stage of the selective process (16–17 generations) using new modern morphometric methods.

In recent years, methods of geometric morphometrics have been widely used in morphometric comparisons (Rohlf & Slice, 1990; Zelditch *et al*., 2004; Klingenberg, 2011), which allow for separate analysis of variability in the size and shape of objects and allow morphogenetic interpretation of detected morphological changes in the configuration of objects (Zelditch *et al*., 2004; Sheets & Zelditch, 2013). The use of 3D geometric morphometrics is most promising for morphogenetic comparisons (Wiley *et al*., 2007; Schlager, 2017; Voyta *et al*., 2021), which allows us to accurately describe changes in the shape of objects, in particular animal skulls. Based on the results of a recent comparison of the skull shape of American and European minks using geometric 3D craniometric methods, which re-

vealed morphological differences between species (Gálvez-López *et al*., 2022), we can use the same methods to assess the level of differentiation of American mink cage strains after selection based on characters of defensive behavior.

The aim of the work is to compare the variability of the centroid size and 3D shape of the skull of aggressive and tame strains of the American mink, formed after long-term selection based on characters of defensive behavior, with a strain of non-selected (control) individuals using geometric morphometrics methods. It was of particular interest to compare the interstrain differences that arose after selection with the degree of sexual dimorphism of cage strains, as well as with their differentiation from the wild Canadian population and another species, the European mink, in order to estimate the levels of their mutual morphological divergence in the common morphospace.

Material and methods

Previously, the American and European mink were classified in the same genus *Mustela* Linnaeus, 1758. Later, after a special study by Abramov (2000), the American mink was assigned to the genus *Neovison* Baryshnikov et Abramov, 1997. Nevertheless, recently, additional studies by Patterson *et al*. (2021) have made it possible to identify four species within the genus *Neogale* Gray, 1865 in North and South America, including the area of the American mink proper, *N. vison*, on the territory of North America. In our study, we adhere to the latest taxonomic decision and the genus name.

The research was carried out exclusively on museum collection material, initially obtained at the experimental fur farm of the Institute Cytology and Genetics, Siberian Branch of RAS. The main material for the work was the skull collection of the American mink created based on the results of experimental selection work led by Dr. O.V. Trapezov (Trapezov, 2012). The experimental strains were obtained after selection carried out over 16–17 generations based on characters of defensive behavior in two directions: aggressive and tame behavior. A sample of non-selected animals, which were kept in parallel (simultaneously) on a fur farm in similar cage management conditions with experimental ones was used as a control. Previously, the studies were approved by the Ethics Committee of the Institute of Cytology and Genetics (Novosibirsk) and performed in compliance with the rules for conducting scientific research using experimental animals approved by the Decree of the Presidium of the USSR Academy of Sciences dated April 2, 1980, No. 12000-496 and the order of the Ministry of Education of the USSR dated September 13, 1984, No. 2.

The analysis included 10 randomly selected individuals of both sexes for three strains — a total of 60 skulls of current year's young of the same age (7 months) born in the same season of the year (November). At this age, individuals of both sexes of the American mink are already approaching mature individuals in many respects in size and proportions (Ternovsky, 1977). In addition to caged animals, a sample of wild American mink from Canada was also studied: 10 skulls of males trapped in Alberta (Wood Buffalo Park, Conibear Lake) in 1933 at the age of 1+ to 3+. The sample was transferred to the Museum of the Institute of Plant and Animal Ecology Ural Branch of the RAS in the late 50s of the XX century during the scientific exchange of collections with the National Museum of Canada. Besides that, the museum skulls of similar-aged adult males of the European mink *M. lutreola* (n = 5), trapped in the Middle Urals in the 50s and 60s of the last century were added.

The overall design of the work included four items of comparison. The first (1) assumed to compare the variation in the skull size based on the centroid (CS). We analyzed male and female samples separately using a One-way ANOVA. Males included besides three cage strains (aggressive, non-selective & tame) a wild Canadian population of American mink and another species — the European mink. A similar comparison was performed for females among only three cage strains of American minks. On the combined material of males and females of the only cage strains of *N. vison*, we evaluated the influence ratio of the factors "strain" (S), "gender" (G) and their interaction (S × G) based on a Two-way ANOVA. The second item (2) was aimed to evaluate the ratio of interstrain and gender differences in the variation of the skull shape. When comparing males and females of only cage American mink strains we used Principal component analysis, canonical analysis, as well as non-parametric multivariate Two-way analysis of variance (PERMANOVA). Under the third item (3) we endeavored to relate intergroup skull shape differences between aggressive and tame cage minks with the degree of their morphological differentiation from non-selected minks, wild Canadian population and the European mink, based on canonical and cluster analyses. The fourth item (4) included an assessment of the morphogenesis instability in each male and female sample of the three cage strains of American mink based on a comparison of the of within-group morphospace volumes (*Vm*) (Vasil'ev, 2021).

Before scanning, the skulls of minks were additionally cleaned of tissues and bleached in hydrogen dioxide (15–20 min). Then the surface of the skulls was coated with antiglare matte spray ATECO WHITE. The skulls were scanned using a Solutionix 3D Rexcan III scanner (South Korea) with an accuracy of 0.01 mm. The resulting STL image files were converted to PLY files. Homologous 33 landmarks were placed on 3D models of skull surfaces in the form of meshes in the Landmark 3.6 program (Wiley *et al*., 2007) (Fig. 1) using only the left side of the skull and its surface, including the line dividing the skull along the sagittal plane.

With the help of the Landmark program, the 3D ordinates of the landmarks for all individuals were placed in a DTA file corresponding to the NTSYSpc format. Further import of the DTA file and analysis of the variation of centroid sizes and shapes of skulls were carried

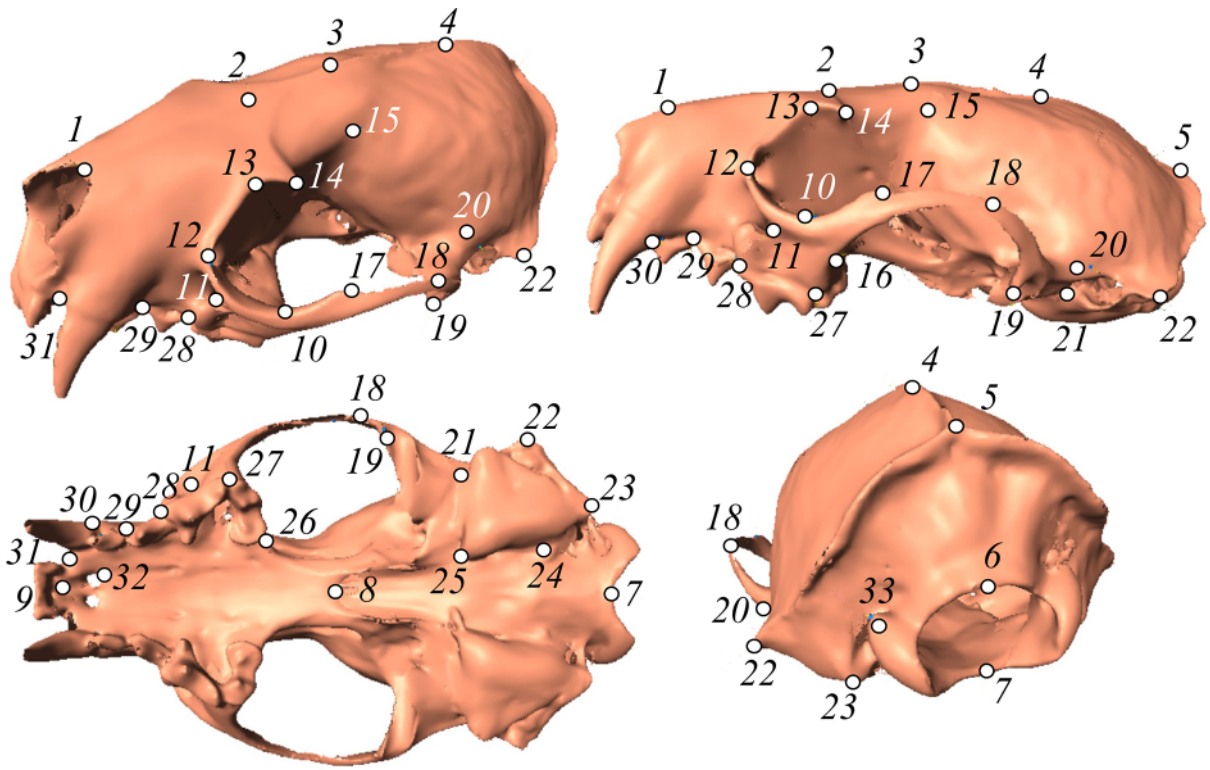

Fig. 1. 3D layout of the landmarks (1–33) on the surface of left the skull side of the American mink (types of landmarks according to F. Bookstein): 1 — cranialmost point between nasal bones (I), 2 — the center of the frontal bone between the supraorbital processes (II), 3 — the middle of the between-orbit space in its narrowest part (II), 4 — the highest height of the skull on the line of the anterior edge of the auditory foramen (III), 5 — mediodorsal tip of the nuchal crest on the side of the occipital foramen (I), 6 — midpoint of dorsal margin of foramen magnum (II), 7 — midpoint of ventral margin of foramen magnum (II), 8 — midpoint of posterior edge of palatine torus (I), 9 — cranialmost medial point of I1 alveolus (I), 10 — the upper edge of the base of the anterior zygomatic process in the area of the largest ventral curvature (II), 11 — the lower posterior edge of the infraorbital foramen (II), 12 — the anterior edge of the orbit below the anterior ocular tubercle of the lacrimal bone (I), 13 — the upper edge of the orbit in front of the base of the supraorbital process (II), 14 — the apex of the supraorbital process (I), 15 — the upper posterior edge of the orbit at the border of the facial and cerebral parts of the skull (III), 16 — a point in the region of the posterior edge of the base of the anterior zygomatic process (III), 17 — the most dorsally curved upper edge of the zygomatic arch on the anterior zygomatic process (I), 18 — the point of the greatest dorsal curvature of the zygomatic arch on the posterior zygomatic process (II), 19 — the anterior edge of the lateral articular tubercle at the base of the posterior zygomatic process (II), 20 — the temporal edge of the orbit at the dorsal part of the base of the posterior zygomatic process (III), 21 — the anterior outer edge of the auditory capsule (II), 22 — the lateral edge of the occipital ridge above the auditory meatus (II), 23 — the posterior lateral edge of the auditory capsule (II), 24 — medial edge of the auditory capsule on the ventral part of the occipital bone at the exit of the left carotid artery (II), 25 — anterior medial edge of the auditory capsule on the side of the basisphenoid bone (I), 26 — the posterior edge of the alveoli of the molar M1 (I), 27 — the lateral point between the alveoli of the predatory tooth Pm4 and molar M1 (I), 28 — the lateral point between the alveoli of the predatory tooth Pm4 and the third premolar Pm3 (I), 29 — the lateral point between the alveoli of the Pm2 and Pm3 premolars (I), 30 — the lateral point between the alveoli of canine C and the Pm2 premolar (I), 31 — the point between the alveoli of the incisor I3 and canine C (I), 32 — the posterior edge of the left incisor foramen (I), 33 — the upper lateral edge of the left occipital condyle on its convex part (II).

out in the MorphoJ 1.7a program (Klingenberg, 2011). Generalized Procrustes Analysis (GPA) was performed using the least squares method (Rohlf & Slice, 1990). The GPA procedure included superimposition of 3D landmark configurations: translation, scaling and rotation, followed by calculation of centroid sizes and Procrustes coordinates. The centroid size (CS) of the skull was estimated as the square root of the sum of the squares of the distances from the center of the image to all landmarks (Rohlf & Slice, 1990). Text files containing the starting and target blocks of the landmark configurations that allow 3D morphing (modeling 3D images of the skull) were obtained based on the results of the Principal Components Analysis (PCA), as well as the Canonical Variate Analysis (CVA) of Procrustes coordinates in the MorphoJ program. The further procedure of 3D morphing objects along these variables is performed in the Landmark program based on the

corresponding meshes (PLY files). As a result, the 3D models of the skull images corresponded to their smallest and largest ordinates along the respective axes (PCA and CVA) were obtained. Additionally, in order to more fully visualize cranial changes, the MorphoJ program also constructed schematic 2D contours of the skull in two, lateral and dorsoventral projections).

The possible effect of allometry — variation in skull shape depending on changes in its size — was assessed based on a regression analysis of PC1 values on centroid sizes (Zelditch *et al*., 2004) separately for each sample of males and females of the three cage strains.

Based on the results of the canonical analysis, the matrix of generalized Mahalanobis distances (*D*) and the matrix of Procrustes distances (*Pd*) between the compared samples were calculated, characterizing the hierarchy of their morphometric intergroup differences. Using the matrix of non-squared generalized Mahalanobis *D*-distances, the mean measure of uniqueness — MMU (by Vasil'ev, 2005) were calculated for each sample, allowing us to assess the mutual proximity and peculiarity of the compared samples in the general morphospace. This indicator actually reflects a measure of morphological diversity (morphological disparity) and allows us to estimate the location of the sample centroid in morphospace relative to other samples.

The Shapiro-Wilk W-test was used to verify that the distribution of variables corresponded to the normal law. The homogeneity of the sample variances was determined using the Levene's test. Multiple paired comparisons were performed on the basis of the post-hoc Tukey's Q-test. In multiple sample comparisons the significance of the differences was assessed using One-way and Two-way variance analyses, as well as the non-parametric multivariate Two-way ANOVA-PERMANOVA (Anderson, 2001).

To assess the degree of sexual dimorphism of CS, the formula $SDM = [(Xf/Xm) - 1] \times 100$ was used, where Xf is the average value of the CS in females and Xm in males (Lovich & Gibbons, 1992). In a multivariate comparison of the skull shape the quadratic generalized Mahalanobis distances (D^2) were used for this purpose with an assessment of their significance levels. In order to obtain a complete picture of the hierarchical relationships between samples and individuals, cluster analysis (UPGMA) of individual values of canonical variables (CV1–CV7) was used, which allows us to assess the stability of the preservation of group morphological specificity in the classification of the skull shape at the level of every individual in the general morphospace. The choice of the most adequate metric for cluster analysis was carried out according to the highest value of the cophenetic correlation coefficient (CCC) proposed by F.J. Rohlf, which was evaluated in the PAST 4.06 program (Hammer *et al*., 2001).

Remind that the concept of "morphospace" as an element of the concept of theoretical morphology, it was most fully presented by J. McGhee (1999). In its interpretation, under the multivariate analysis of the variation each individual ordinate is a point in the general morphospace. To assess the within-group level of instability of morphogenesis we used the *Vm* indicator — the volume of the within-group morphospace occupied by the ordinates of this group (Vasil'ev, 2021). The volume of the within-group morphospace enclosed inside the convex hull — *convexhull* (Cornwell *et al*., 2006; Blonder, 2018), constructed from a set of external marginal ordinates of objects, we estimated using the values of three canonical variables (CV1–CV3) calculated from Procrustes coordinates characterizing the shape variation of the skull. Since the samples aligned by the number of observations are compared, the *Vm* indicator allows us to obtain comparable characteristics of the scattering values of the ordinates of individuals in the morphospace. The greater the value of *Vm*, the less stable the development of a group of individuals is and to a greater extent there is a scatter of their ordinates in the morphospace, indicating an expansion of the set of morphogenetic trajectories (Vasil'ev, 2021). In more favorable conditions, i.e. with minimal stress during development, the value of the *Vm* indicator is less than under adverse conditions.

To calculate the volumes of the within-group morphospace (*Vm*), the add-in Calculate Volume (author A.G. Kursanov) for Microsoft Office Excel was used, written on the basis of the built-in MatLab *convhull* function, which allows calculating the volume of the convex hull of a finite set of points (3D convex hull). To calculate the volume of within-group morphospace — *Vm*, we can also recommend the *hypervolume* R program (Blonder, 2019). When estimating the standard measurement error *Vm* (±SE), the bootstrap technique with random re-substitution of objects in the sample was used (Efron & Tibshirani, 1986). Multiple comparisons of samples by *Vm* values were carried out based on non-parametric analog of One-way analysis of variance — the Kruskal-Wallis H-test. Calculations were performed using TPS application software packages (Rohlf, 2017a, b), PAST 4.06 (Hammer *et al*., 2001), Landmark 3.6 (Wiley *et al*., 2007), MorphoJ 1.07a (Klingenberg, 2011).

Results

1. The skull size variation: between-strain, sexual, "cage–wild" and interspecies aspects

CS values in males of the three cage strains, the wild Canadian population of the American mink and the European mink are shown on Figure 2.The Levene's test, based on means, showed that the variances of the compared samples are homogeneous ($p = 0.3093$). Shapiro-Wilk test revealed no deviations from the normal distribution law for residuals ($W = 0.9739$, $p = 0.3985$). According to the results of a one-way ANOVA, there are significant differences among males ($F = 19.19$; d.f. = 4, 40; $p < 0.0001$). When comparing in pairs, the skull centroid sizes of the European mink is significantly smaller than those of all samples of the American mink (in all pairs: $p < 0.001$). Males of the cage strains of aggressive and non-selective American

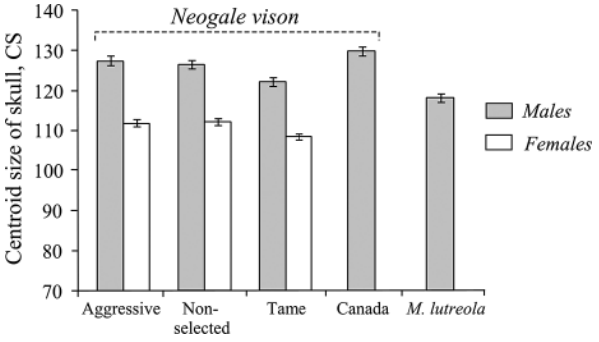

Fig. 2. Comparison of mean centroid sizes CS (with standard errors ± SE) among males and females of aggressive, non-selected and tame strains, wild Canadian males of the American mink, as well as in the European mink males.

minks do not differ neither from each other nor from males of the wild Canadian population (respectively, Q = 1.10, $p = 0.9357$; Q = 2.29, $p = 0.4960$; Q = 3.39, p = 0.138). At the same time, the smallest tame strain significantly differ from all other samples of American mink males (from $p = 0.0287$ to $p = 0.0002$).

Similar analysis among females of three cage strains also revealed significant differences between them ($F = 12.34$; d.f. = 2.27; $p < 0.00016$; Levene's test: $p = 0.2735$), which were also due to significantly lower CS value in tame females (compared with aggressive Q = 5.54, $p = 0.00166$ and non-selected Q = 6.52, $p = 0.00036$). Differences in CS between females of aggressive and non-selected minks have not been statistically confirmed (Q = 0.98, p = 0.7690).

Significant differences were also revealed between the samples of males and females of three cage strains in terms of cranial CS values ($F = 102.3$; d.f. = 5, 54; $p < 0.00001$; Levene's test: $p = 0.2346$). In all pairs of comparison significant differences were found between males and females, in terms of CS (the values of Q in Tukey's test pairwise comparisons range from Q = 12.08 to Q = 23.32 with equal levels of significance: $p = 0.00138$): in all cases the skull size in males is larger than that of females.

The highest index of sexual dimorphism was manifested in aggressive individuals (SDM = 12.83%), and the lowest in tame (SDM = 10.71%). An intermediate index value was obtained for non-selected minks (SDM = 11.17%). In all strains, the values of the SDM index are close in level and indicate larger CS values in males compared to females.

Since two factors: "strain" (S) and "gender" (G) appear in the intergroup comparison of CS values among of American mink samples, we conducted a Two-way ANOVA of the CS values among samples of males and females in these strains, taking into account the interaction of factors (S × G) (Table 1). From the table it follows, that the influence of the S factor is minimal, but statistically significant (the proportion of intergroup variance in this case was 3.07%). At the same time, the influence of the G factor on the variability of CS turned out to be predominant (the proportion of variance was 82.49%). The interaction of factors is negligible and statistically insignificant ($p = 0.5140$).

2. Skull shape variation in cage American minks: sexual and interstrain differences

2.1. Principal Components Analysis

In the Figure 3 it can be seen that along the first Principal component (PC1), which accounted for about 64.2% of total variance, sexual differences appeared: on the left of the graph there are centroids of samples of females of the three strains being compared, and on the right — males. Along the PC2, which characterizes about 21.7% of total variance of the 3D shape of the skull, mainly interstrain differences appeared: the low PC2 values were on the centroids of samples of aggressive males and females, and the high — on the centroids of tame individuals of both sexes. Non-selected males and females occupy an intermediate position, however, if the female sample centroid of this strain is localized in the morphospace closer to the sample of tame females, then male one is closer to the aggressive strain.

Finally, along PC3 (9.7% of total variance), both males and females show a tendency to differ between both selected strains on the one hand and the non-selected strain (their centroids have the lower values), on the other. Along PC3 the "strain × gender" interaction also manifests itself, since the sex centroid ordinates are expressed to various degree in different strains.

The Fig. 3 shows graphical contour 2D configurations of skulls in two projections (lateral and dorsoventral) as well as 3D models of the skull shape obtained

Table 1. The results of a Two-way ANOVA of centroid sizes (CS) of males and females of aggressive, non-selected and tame American minks, taking into account the influence of factors "strain" (S), "gender" (G) and their interaction (S × G).

The source of variation (factor)	Sum of squares (SS)	Degree of freedom (df)	Mean square (MS)	F	Significance level (p)
Strain (S)	117.50	2	58.74	5.88	0.0049
Gender (G)	3157.00	1	3157.00	316.30	< 0.0001
Interaction (S × G)	13.45	2	6.73	0.67	0.5140 (ns)
Residual	539.00	54	9.98		
Total	3826.95	59			

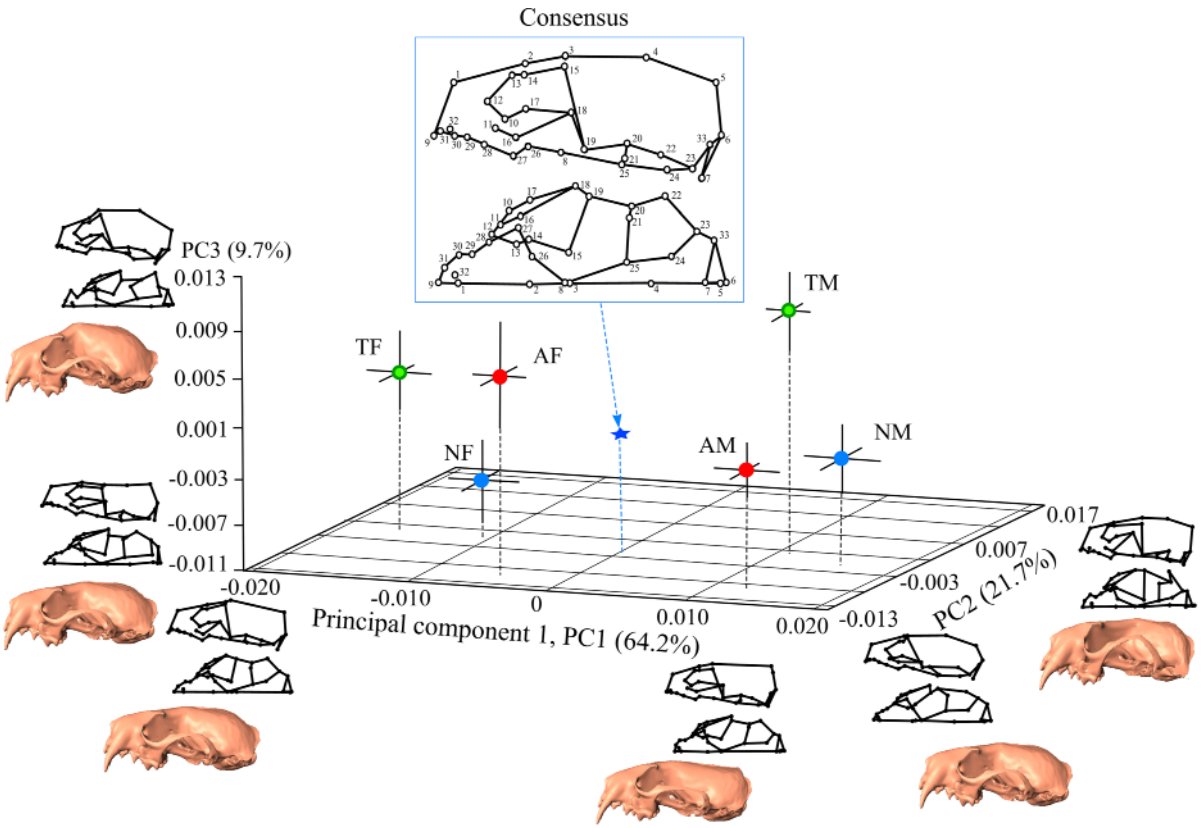

Fig. 3. The results of the Principal Components Analysis (PCA) of the 3D skull shape among males (M) and females (F) of aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) strains of American mink in the general morphospace of the first three axes (PC1–PC3), with standard errors (±SE) of the sample centroids. The 3D skull morphing models and their wire-frame configurations in two projections (lateral, dorsoventral) for the minimum and maximum PC values are presented along the axes. The exaggeration factor is 3.0.

during the morphing procedure, corresponding to the maximum and minimum values of the respective PC. The male skulls are generally more elongated and flattened, and their facial part is somewhat elongated, with the canines pointing at a relatively large angle in the anterior direction. The females exhibit opposite structural features. Tame males and females demonstrate pronounced narrowing of the skull in the area of the between-orbit interspace with a simultaneous increase in the height of the skull in the area of the frontal bones (bulges of the dorsal central part of the cranial vault), as well as some relative shortening of the facial part.

As a result of regression analysis of the variation in PC1 values on CS, obtained separately for samples of males and females of each cage strain, in no case did we identify significant allometric dependences of shapes on sizes ($0.2034 < p < 0.7783$).

2.2. Canonical Variates Analysis

Table 2 shows the mean values of the centroids of six samples of cage American minks, taking into account standard errors and formal statistical estimates obtained as a result of the canonical analysis. Based on the signs of the centroids, it is possible to interpret the intergroup differences. Along the first canonical axis (CV1), the greatest differences in the values and signs of the centroids appeared between samples of different sexes: the male centroids lie in the interval of negative values, while the female ones are shifted to the positive direction. This is clearly seen in Figure 4, where images characterizing the extreme deformations of the skull along the first three canonical variables CV1–CV3 were constructed using the 3D morphing procedure carried out following the results of the canonical analysis. Indeed, along the first canonical variable CV1, as in the case of the PC analysis of the of these groups, sexual differences are expressed: in the region of the minimum values of CV1, the ordinates of males of all three strains are localized in the morphospace, and in the region of the highest values, the corresponding ordinates of female groups. Along the CV2 and the CV3 interstrain differences appeared between the strains of aggressive and tame American minks (Fig. 4, Table 2). The minimum CV2 values correspond to the ordinates

Table 2. The results of the Canonical Variates Analysis (CVA) of the Procrustes coordinates in experimental and control samples of the American mink.

Strain & gender	Canonical variables		
	CV1 ± *SE*	CV2 ± *SE*	CV3 ± *SE*
The sample centroids			
Aggressive male, AM	–2.567 ± *0.246*	–2.865 ± *0.162*	–0.792 ± *0.301*
Aggressive female, AF	3.122 ± *0.266*	–3.185 ± *0.382*	–1.696 ± *0.315*
Non-selected male, NM	–5.367 ± *0.403*	0.104 ± *0.220*	0.710 ± *0.379*
Non-selected female, NF	4.620 ± *0.387*	–0.340 ± *0.517*	3.512 ± *0.228*
Aggressive male, AM	–3.082 ± *0.297*	2.768 ± *0.177*	0.183 ± *0.312*
Aggressive female, AF	3.873 ± *0.275*	3.478 ± *0.301*	–2.038 ± *0.354*
Results of canonical analysis			
Wilks' Λ-test	0.00015	0.00271	0.02143
Eigenvalue	16.6614	6.9638	3.7639
Canonical correlation coefficient	0.97	0.94	0.89
Proportion of variance, %	52.22	21.83	11.80
Chi-squared test (χ2)	470.22	316.60	205.60
Degree of freedom (df)	25	16	9
Significance level (*p*-value)	< 0.00001	< 0.00001	< 0.00001

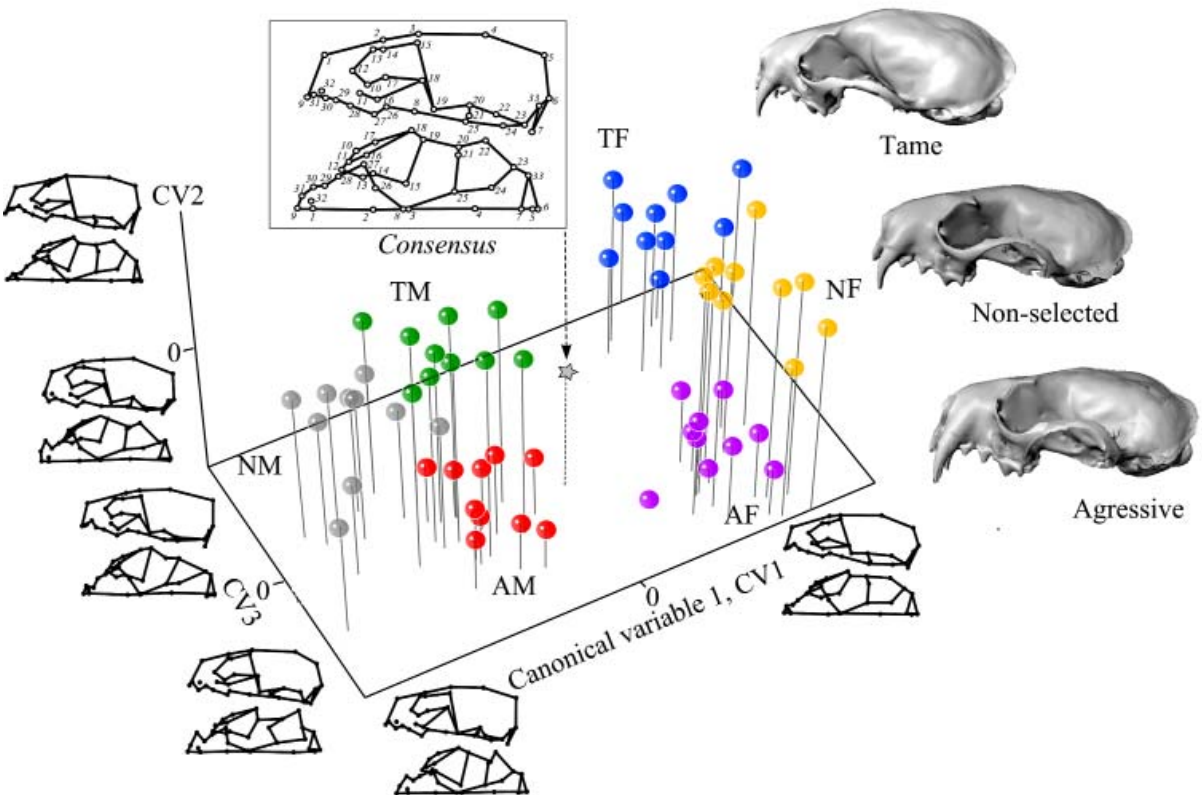

Fig. 4. The results of the Canonical Variate Analysis (CVA) of the 3D skull shape among males (M) and females (F) of aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) strains of American mink in the general morphospace along three canonical axes (CV1–CV3). The 3D skull morphing models and their wireframe configurations in two projections (lateral, dorsoventral) for the minimum and maximum CV values are presented along the axes. The exaggeration coefficient is 3.0.

Table 3. The results of a non-parametric multivariate Two-way analysis of variance (PERMANOVA) of canonical variables (CV1–CV5) characterizing the intergroup variation of the skull shape among males and females of aggressive, non-selected and tame American mink strains, taking into account the factors strain (S), gender (G) and their interaction (S × G).

The source of variation (factor)	Sum of squares (SS)	Degree of freedom (df)	Mean square (MS)	F	Significance level (p)
Strain (S)	583.21	2	291.61	58.32	< 0.0001
Gender (G)	933.59	1	933.59	186.72	< 0.0001
Interaction (S × G)	291.01	2	145.50	29.10	< 0.0001
Residual	270.00	54	5.00		
Total	2077.81	59			

of aggressive males and females, and the maximum values correspond to the ordinates of tame individuals of both sexes.

The ordinates of non-selected strain, both males and females, occupy intermediate position between the aggressive and tame strains. Thus, on the 3D models of contour skull images located at the edges of the axes characteristic phenotypic differences of all three strains can be seen. In particular, tame American minks show an increase in the relative height of the skull in the frontal bone area, a relative shortening of the facial part, an increase in the eye socket and a decrease in the auditory capsules. The aggressive minks have other structural features that are opposite to tame ones: a large elongation and flattening of the skull, smaller eye sockets with a wider between-obit interspace, relatively large canines, as well as auditory capsules. Along the CV3, interstrain differences were also clearly manifested. The ordinates of the three compared strains for males and females are arranged in parallel rows: aggressive individuals are grouped in the area of the lowest CV3 values, and tame ones in the area of the largest (Fig. 4). The non-selected minks along CV3 are projected closer to the middle of the axis and have intermediate features of the skull configuration compared to aggressive and tame individuals.

In order to relate the influence of strain (S) and gender (G) factors, as well as to take into account the factor interaction (S × G) on the variability of the skull shape in cage American minks of all three strains, a non-parametric multivariate Two-way analysis of variation (PERMANOVA) was performed for all five canonical variables. The results of the analysis are presented in Tab.3. Both factors and their interaction had a statistically significant effect on the variability of the skull shape. The greatest contribution to the variation of the skull shape was made by the gender of individuals (the proportion of variance was 49.48%). On the second place in terms of the degree of influence of the factor were the interstrain differences associated with the selection of individuals for defensive behavior (the proportion of variance was 29.65%) — factor "strain." The interaction of factors affects variability to a lesser extent (the proportion of variance is 11.87%), but it is also an essential component of the intergroup variance, reflecting the different effects of selection on males and females of different strains. The proportion of residual variance is only 9%.

3. Cage effect and interspecies differences

The results of the canonical analysis of the skull shape of in American mink males (experimental and control cage strains) in comparison with a sample of males from the wild Canadian population of the species and males of the European mink are shown in Figure 5. Intergroup differences along all canonical variables are statistically significant ($p < 0.0001$). The first three canonical axes accounted for 80.01% of the variance (Table 4), which allows us to characterize the intergroup differences rather fully. CV1 (46.77%) demonstrates interspecies differences as well as sexual ones. Figure 5 clearly shows that the differences between the ordinates of the experimental strains are large, but evenly between the extreme ones — aggressive and tame — they are less pronounced than the mutual farness of American and European mink samples in the morphospace. The ordinates of males from the wild Canadian population of the American mink are shifted from the cage individuals along CV2 to the range of its lower values. However, Canadian individuals are closest to the ordinates of males of aggressive and non-selective American minks. The wild Canadian sample is shifted along CV3 to the direction of its lowest values and manifests itself in morphospace as an analogue of another differentiated "strain" in relation to samples of males of three cage strains. The differences in the configurations of skull 3D models between experimental strains in the Fig. 5 correspond to those in Fig. 4. The skulls of the European mink, unlike the American one, are more elongated and flat with a relatively enlarged eye socket and are characterized by a relatively smaller size of canines.

The matrix of generalized Mahalanobis distances (D) (Table 5) allows us to assess the scale and hierarchy of intergroup differences based on the results of this canonical analysis. The greatest differences in skull shape appeared between the compared species: American and European minks. Approximately a similar level of sexual differences was manifested in aggressive ($D^2 = 76.91$; $F = 82.28$; $p < 0.00001$) and tame ($D^2 = 92.35$; $F = 90.43$; $p < 0.00001$) strains of the American mink, but in the non-selected strain, the dif-

Table 4. The results of the Canonical Variates Analysis (CVA) of the Procrustes coordinates characterizing the variation of the skull shape among samples of cage and wild American mink, as well as the European mink.

Sample & gender	Canonical variables		
	CV1 ± *SE*	CV2 ± *SE*	CV3 ± *SE*
The sample centroids			
American mink:			
Aggressive males, AM	–5.023 ± *0.270*	0.225 ± *0.364*	3.730 ± *0.326*
Aggressive females, AF	2.821 ± *0.376*	3.420 ± *0.257*	4.293 ± *0.261*
Non-selected males, NM	–3.312 ± *0.371*	–1.571 ± *0.441*	–3.498 ± *0.373*
Non-selected females, NF	2.740 ± *0.335*	4.411 ± *0.249*	0.945 ± *0.338*
Aggressive males, AM	–7.076 ± *0.256*	0.654 ± *0.266*	–4.665 ± *0.197*
Aggressive females, AF	1.216 ± *0.301*	4.955 ± *0.205*	–1.472 ± *0.387*
Canada, males, CanM	–2.231 ± *0.234*	–9.835 ± *0.336*	2.225 ± *0.289*
European mink:			
Mustela lutreola, males, *Mlut*M	21.738 ± *0.554*	–4.520 ± *0.536*	–3.110 ± *0.449*
Results of canonical analysis			
Wilks' Λ-test	0.00000005	0.00000285	0.00007325
Eigenvalue	51.40377	24.71842	11.82048
Canonical correlation coefficient	0.99	0.98	0.96
Proportion of variance, %	46.77	22.49	10.75
Chi-squared test (χ2)	1112.39	849.12	633.18
Degree of freedom (df)	49	36	25
Significance level (*p*-value)	< 0.00001	< 0.00001	< 0.00001

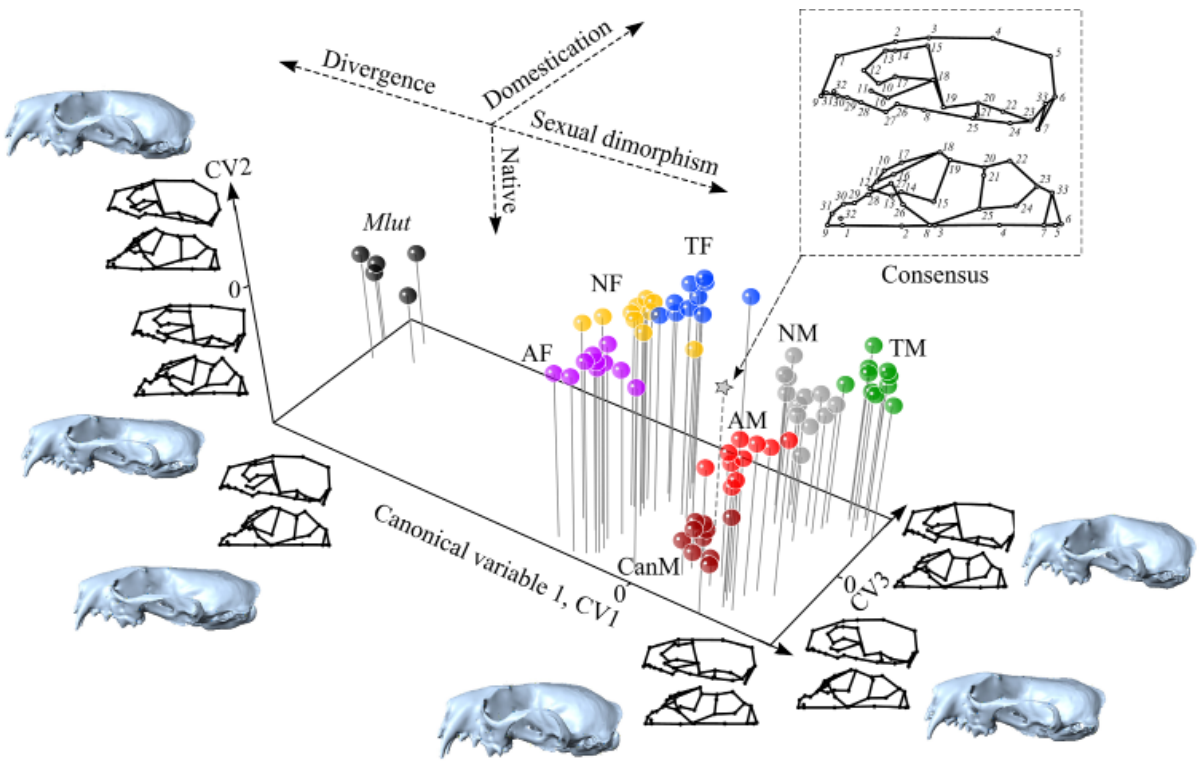

Fig. 5. The results of the Canonical Variates Analysis (CVA) of the 3D skull shape among samples of aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) strains, wild Canadian males of the American mink (CanM), as well as the European mink males (*Mlut*M) in the general morphospace along three canonical axes (CV1–CV3). The 3D skull morphing models and their wireframe configurations in two projections (lateral, dorsoventral) for the minimum and maximum CV values are presented along the axes. The exaggeration coefficient is 3.0.

Table 5. Matrix of generalized Mahalanobis distances (*D*) among samples of males (M) and females (F) of the cage aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) strains, as well as wild Canadian population of the American mink (CanM) and the wild European mink (*Mlut*M) with MMU (mean measure of uniqueness) values.

Species, strain & gender	*Neogale vison*							*Mustela lutreola*	MMU
	Aggressive		Non-selected		Tame		Wild	Wild	
	AM	AF	NM	NF	TM	TF	CanM	*Mlut*M	
AM	0	****	****	****	****	****	****	**	11.39
AF	8.77	0	****	****	****	****	****	***	10.88
NM	7.89	12.15	0	****	****	****	****	***	12.54
NF	10.64	6.44	12.36	0	****	****	****	***	11.01
TM	9.35	12.06	7.71	10.58	0	****	****	**	12.17
TF	11.39	8.021	12.40	5.96	9.61	0	****	**	11.17
CanM	10.85	10.86	12.25	13.01	13.57	12.24	0	**	13.26
*Mlut*M	20.86	17.88	23.00	18.08	22.30	18.55	20.01	0	20.10

Note:** — $p < 0.01$; *** — $p < 0.001$; **** — $p < 0.0001$.

ferences between males and females were more pronounced ($D^2 = 152.77$; $F = 99.55$; $p < 0.00001$). The latter can be seen also in Figure 4, where the ordinates of the samples of males and females of the strain of non-selected animals occupy the extreme along CV1 — the largest and smallest values.

Table 5 also shows the values of the mean measure of uniqueness — MMU. Females in different cage strains of American minks, compared with those of other strains, have the lowest values of the MMU index and, therefore, occupy a central position in the general morphospace. The differentiation among males of the compared strains in terms of MMU values is more pronounced than among females. The highest average measure of uniqueness (analogous to morphological disparity) appeared, as expected, in the European mink (MMU = 20.10). Judging by the magnitude of the MMU index, wild Canadian males occupies an intermediate position in the morphospace between the European and American minks, but is placed significantly closer to the latter.

We do not provide a matrix of Procrustes distances (*Pd*) between the compared samples, since there was a significant strong correlation between the values of this matrix and the matrix of generalized Mahalanobis distances (Mantel's R = 0.91, $p = 0.0002$).

The results of cluster analysis (UPGMA) at the individual level according to the ordinate values of all canonical variables are presented in Fig. 6. In fact, they reflect the hierarchy of manifestation of a combination of individual and intergroup variations of all compared samples of American and European minks. The figure clearly shows that two main clusters belonging to two species have been identified. In the cluster of the American mink, a separate, most differentiated clade includes wild Canadian males (CanM). In turn, American mink was divided into subclusters of males and females. In females, as in males, the branch belonging to the aggressive strain is more differentiated, and the tame and non-selected minks are relatively closer in morphospace. All the compared individuals, without exception, were unmistakably divided by belonging to their own samples.

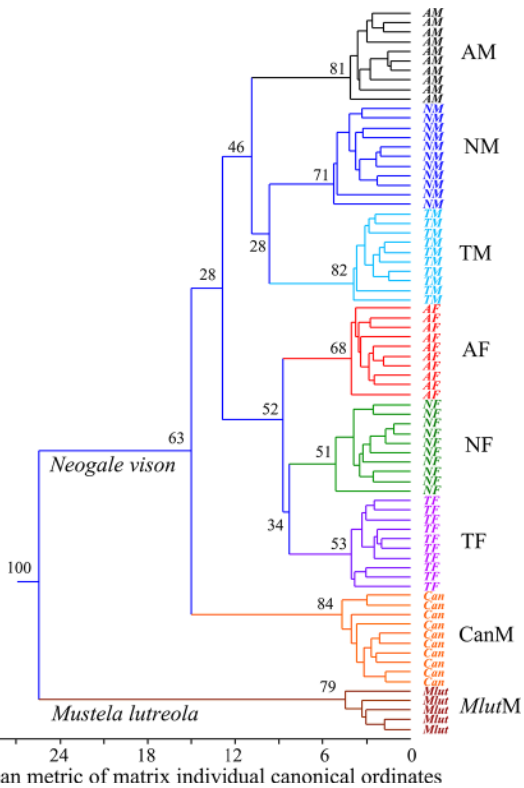

Fig. 6. The results of cluster analysis (UPGMA) in the Euclidean metric of a rectangular matrix of individual values of CV1-CV7 among samples of aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) strains, wild Canadian males of the American mink (CanM), as well as in the European mink males (*Mlut*M)). Bootstrap support at cluster main nodes is indicated, %.

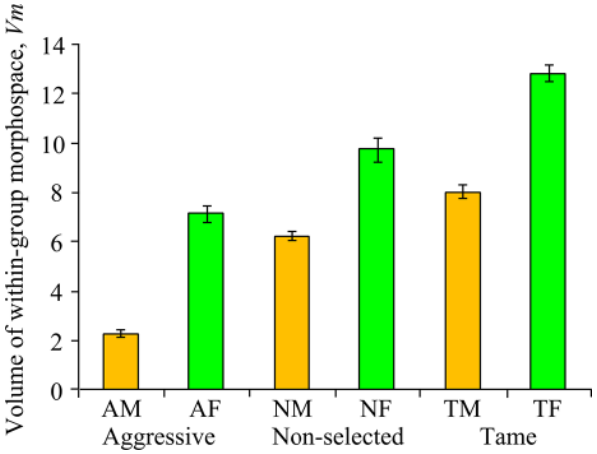

Fig. 7. Comparison of the within-group morphospace volumes *Vm* (with standard errors ± SE) among males (M) and females (F) of aggressive (AM, AF), non-selected (NM, NF) and tame (TM, TF) American mink strains.

4. Within-group morphospaces volumes in cage strains of American mink as a measure of development instability

Resulting from the comparison of the volumes of within-group morphospaces (*Vm*), in samples of males and females of three cage strains of the American mink based on resampling (Fig. 7), significant sexual differences were revealed. In all strains, females demonstrate a significantly larger volume of within-group morphospace compared to males (for aggressive ones — $Q = 15.34$, $p < 0.0001$; for non-selected ones — $Q = 11.17$, $p < 0.0001$; for tame ones — $Q = 15.31$, $p < 0.0001$). At the same time, significant interstrain differences were also manifested (Kruskal-Wallis $H = 26.74$, $p < 0.0001$): the lower values were observed in the aggressive strain, and the higher ones in the tame strain (see Fig. 7). The highest *Vm* was found in females in the tame mink strain. An intergroup comparison of *Vm* based on the non-parametric Kruskal-Wallis H-test revealed significant differences in males of three strains ($H = 24.82$, $p < 0.0001$), as well as in a similar comparison of females ($H = 22.81$, $p < 0.0001$).

The results of Two-way ANOVA in assessing the ratio of the shares of the influence of the factors "strain" (S),"gender" (G) and their interaction (S × G) on the variation of *Vm* values in groups of males and females of the compared cage strains of the American mink are presented in Table 6. The influence of the factors "strain" and "gender" on the variation in the volume of within-group morphospaces (*Vm*) turned out to be statistically significant. The proportions of the contribution of two factors to the intergroup variance are close in magnitude, but the influence of interstrain differences, nevertheless, seems to be slightly higher (proportion of variation — 48.54%) than for the "gender" factor (42.71%). The effect of the interaction (S × G) on the variability of *Vm* values was not statistically confirmed ($p = 0.0651$).

Discussion

Since, according to the centroid size of the skull, the effects of selection on characters of defensive behavior in American minks were weakly manifested, expressed only in a slight increase in the size of the skull (according to CS values) of aggressive ones and its same decrease in tame minks, it can be concluded that the size are poorly affected by selection. However, it is known that when comparing American minks by skull measurements from natural populations and from a fur farm in the Tver Region of the Russian Federation, the animals of the fur farm differed in somewhat larger sizes, which the authors attributed to the effect of breeding (Korablev *et al*., 2018). At the same time, it was assumed that the size of individuals, which determines the size of the skins obtained, creates a commercial incentive for this direction of breeding. In another study conducted in Canada, the uniqueness of the wild populations of American minks and individuals of the species kept on animal farms allowed the authors to identify animals that escaped from the farm in nature by the size of their skulls (Tamlin *et al*., 2009).

It should be noted that there is some general tendency to increase the size of minks kept in farm conditions, noted earlier by Korablev *e al*. (2018) was not formally confirmed in our material, perhaps due to the fact that our animals are somewhat younger. It can be assumed that if we compared fully aged natural minks and individuals of an experimental fur farm, the skull size of linear American minks could increase slightly

Table 6. Two-way ANOVA of the within-group morphospace volumes (*Vm*) along the first three canonical variables (CV1–CV3) in assessing differences in the skull shape among samples of males and females of aggressive, non-selective and tame American mink strains, taking into account the factors "strain" (S), "gender" (G) and their interaction (S × G).

Sources of variation (factor)	Sumof squares (SS)	Degree of freedom (df)	Mean square (MS)	*F*	Significance level (*p*)
Strain (S)	327.10	2	163.60	165.60	<0.0001
Gender (G)	287.80	1	287.80	291.50	<0.0001
Interaction (S × G)	5.68	2	2.84	2.88	0.0651 (ns)
Residuals	53.33	54	0.99		
Total	673.91	59			

and, perhaps, even exceed the size of representatives of the natural population. At the same time, since the current year's young from the strains of the American mink that we had at our disposal showed characteristic sexual differences and ridges formed on the dorsal side of the skull, they phenotypically look quite mature. This is also indicated by the similarity of non-selected and aggressive males in terms of CS size with a sample of males of wild Canadian individuals. Therefore, at the moment, it is difficult to judge from our material whether the size of the skull of American minks increased during prolonged maintenance in a fur farm, as noted in a number of the above-mentioned works, or this did not happen.

The sex differences in the size of the skull in our comparison were clearly manifested, which is not surprising and typical for this species, as well as many other species of mustelids (Abramov & Tumanov, 2003; Loy *et al*., 2004; Korablev *et al*., 2013). In our comparison, the sex differences in the 3D skull shape of the American mink also came to the fore and are second only to interspecies ones when compared with the European mink. Unfortunately, we had only a sample of males of the European mink at our disposal, which did not allow us to assess the level of sexual differences in it. Earlier, in the work (Gálvez-López *et al*., 2022) it was shown that if the American mink from Spain clearly shows sexual differences in size and shape of the skull, then the European mink in this territory did not show sexual dimorphism in these relations. However, in the North-East of Europe, sexual dimorphism was detected in the European mink by the skull size (Abramov & Tumanov, 2003). It can be assumed that in different biotic and climatic conditions and with different intrapopulation demographic situations, sexual dimorphism in the European mink, as well as in the American mink, can manifest itself to varying degrees due to niche divergence (Law & Mehta, 2018) and phenotypic plasticity (Schlichting & Wund, 2014). Indirectly, this is indicated by the case of significant differences in the skull shape between males and females of the non-selected strain of the American mink (see the Fig. 4) and, on the contrary, a slight decrease in the sexual differences in the skull shape in the of aggressive strain.

It is important to emphasize that in none of the samples of cage American minks were found cases of allometry. Therefore, all the intergroup differences we identified are not due to allometry effects.

Recall that the relatively recently described cellular mechanism of domestication syndrome (Wilkins *et al*., 2014) and related embryonic, morphogenetic and morphological effects (Lord *et al*., 2020) were established mainly by analyzing the manifestations of domestication in canine, silver foxes (Belyaev & Trut, 1989; Trut *et al*., 2021), rats (Plyusnina *et al*., 2011; Sing *et al*., 2017) and a number of other domesticated animal species (Lord et al., 2020). Such studies have not yet been conducted on the American mink, which has been subjected to long-term selection for characters of defensive behavior.

Our analysis allows us to conclude that indirect traits of domestication syndrome have also appeared in this species. In this regard, it should be noted that in the tame males and females studied by us, there was a pronounced narrowing of the skull in the area of the between-orbit interspace with a simultaneous increase in the height of the skull in the area of the frontal bones (bulge of the dorsal central part of the cranial vault), as well as a tendency to relative shortening of the facial part and some decrease in canines.

As a result of selection based on characters of defensive behavior, morphogenetic effects were achieved in a relatively short period, which are comparable, and in some strains even exceed the differences between wild minks and individuals from a fur farm, the history of keeping those in captivity lasts more than a century. However, the morphological evasion of cage American minks from wild Canadian individuals is significantly less compared to the extent of interspecific differences. If we assume the greatest morphometric distance between males of *N. vison* and *M. lutreola* for 100% (see the Tab. 5), then the level of differentiation between wild and cage minks will be almost half of this value, and the range of differences between the aggressive and tame strains is 41%. It is noteworthy that selection to enhance characters of aggressive behavior led to the recreation of some phenotypic characteristics typical of wild individuals in minks from a fur farm and brought these groups closer together in the general morphospace. Interestingly, the highest degree of sexual dimorphism in the skull shape, revealed in non-selected individuals, is approximately 54% of the range of interspecific differences. However, the revealed morphogenetic effects of selection based on characters of defensive behavior between the strains of aggressive and tame minks and at the intraspecific level turned out to be quite large in scope. They are only slightly inferior to the high level of sex differences characteristic of the species, and are also comparable to the degree of morphological changes that occurred in wild minks when they were kept on fur farms in different countries. The high level of differentiation of the aggressive and tame strains of minks is also confirmed by high-precision and effective recognition of the belonging of each individual to its own strain and sex group during canonical and cluster analyses.

Samples of wild American minks from the Canada and another species, the European mink, make it possible not only to assess the relative scale of interstrain differences, but also the hierarchy of relations between individual ordinates in an expanded morphospace. The results of canonical and cluster analyses reflect stable intergroup morphogenetic differences with high taxonomic weight. The well-known "criterion of 75%" (Mayr, 1969) is currently practically not used, but it can serve as a kind of quantitative threshold level. Since the discrimination of samples of experimental strains reached 100%, exceeding the Mayr's criterion, it can be assumed that the morphological differences between representatives of these strains are very large.

There was a morphological hiatus in the morphospace between the experimental strains of cage minks, therefore, according to the degree of manifestation of intraspecific morphological differences, the strains of aggressive and tame minks formally approached the subspecies rank (of course, we are not talking about the formation of new subspecies during selection) and thereby demonstrate the microevolutionary nature of the morphogenetic changes that occurred.

Based on the results of a Two-way ANOVA of *Vm* values, it can be concluded that the destabilization of the morphogenesis of the skull of American minks, manifested in males and females of different cage strains, is primarily associated with interstrain differences, i.e. determined mainly by the results of selection based on characters of defensive behavior, and sexual differences in this respect are somewhat inferior. It is important to emphasize that in tame individuals of the American mink, an increase in the volume of within-group morphospace (*Vm*) was revealed, but at the same time, a decrease in the *Vm* index is characteristic for aggressive ones. If the first case can be interpreted as a general effect of increased destabilization of skull morphogenesis in the strain of tame minks, then in the latter case, one of the species modifications of development is probably fixed as a result of selection of individuals based on characters of aggressive defensive behavior.

Thus, selection based on opposite features of defensive behavior led to opposite morphogenetic effects: increased development destabilization in males and especially in females of the tame strain and, conversely, constraints of morphogenetic variability in aggressive one. These results are in good agreement with the theory of destabilizing selection by Belyaev (Belyaev, 1979; Belyaev & Trut, 1989), i.e. indicates the possibility of increasing variability and the emergence of new phenotypic properties due to selection based on behavioral traits. On the other hand, the apparent stabilization of skull morphogenesis in the aggressive strain of minks rather reflects the forced nature of constraining the variability that arose when fixing one of the previously historically arisen developmental pathways. In some periods of the population's life, this variant of development as a special modification could provide adaptive advantages due to the realization of the phenotypes of aggressive individuals. Therefore, the case of reducing and limiting ("fixing") the variability of the skull shape in the strain of aggressive minks can also be considered as a special variant of destabilization of development, i.e. as an exhaustion resulting from selection based on the characteristics of behavior, the initially high potential of phenotypic variability, which, in particular, has been preserved in the strain of non-selected minks.

In recent decades, it has been repeatedly shown that microevolutionary rapid rearrangements of morphogenesis can be based on epigenetic changes and their transgenerational inheritance (Jablonka & Raz, 2009; Burggren, 2016; Donelan *et al*., 2020). Therefore, it can be assumed that the relatively rapid progressive morphogenetic responses of experimental American mink strains to selection based on characters of defensive behavior are due to stress-induced epigenetic changes associated with morphogenesis rearrangements, which could be preserved and accumulated during the reproduction of strains in subsequent generations. There are proven examples of transgenerational epigenetic effects on animal behavior (Jensen, 2013). All this can lead to rapid fixation of changes in behavior and morphogenesis. This hypothesis can be potentially verified by molecular genetic methods when comparing the epigenetic profiles of DNA methylation in representatives of experimental strains and control (non-selected) minks at the experimental fur farm of Institute of Cytology and Genetics of Siberian Branch of RAS. If the hypothesis about the epigenetic mechanism of rapid selective response of strains is confirmed, there will be grounds to believe that it is based on the reproduction and enhancement of morphogenetic modifications that previously historically arose in the species, leading to characteristic phenotypes of aggressive and tame individuals. As a result of special selection based on characters of defensive behavior these latent developmental modifications were probably identified, picked up and strengthened, which may to explain a relatively rapid morphogenetic divergence of experimental strains.

Conclusion

A comparative study of the size and shape skull variations in American mink strains obtained in an experimental fur farm as a result of long-term selection based on characters of defensive behavior, we can conclude the following. Selection led to significant morphogenetic changes in both experimental cage strains, both in comparison with each other and with the strain of non-selected individuals, which was considered as a control. The level of phenotypic differences between aggressive and tame minks is comparable to the level of differentiation between the non-selected cage strain of the American mink from and the wild Canadian conspecific population. However, if the change in the morphogenesis of wild minks occurred after keeping in captivity of almost a century, then the selection period of the aggressive and tame mink strains formation was relatively short. We have shown that the degree of divergence between aggressive and tame minks in terms of skull shape was about half of the interspecific differences when compared with another species — the European mink. Judging by the results of a morphometric comparison of two experimental strains of the American mink, a clear morphological hiatus in the skull shape appeared between them. It formally corresponds to the subspecies rank of differences, i.e., their divergence demonstrates, thereby, the microevolutionary nature of morphogenetic changes.

It is important to note that selection had little effect on the skull size, although the tame strain as a whole showed a slight tendency to decrease them. However, the selective process led to a significant divergence of aggressive and tame individuals in the skull shape. Ag-

gressive animals, both males and females, diverged to a greater extent according to the skull configuration. They are distinguished by a more massive and elongated facial part of the skull and relatively larger canines. Tame individuals acquired other characteristic features — swelling of the skull in the frontal bone area, narrowing of the between-orbit interspace, relative enlargement of the eye socket, a tendency to shorten the facial part and decrease the canines. The latter features may be associated with the manifestation of the embryonic mechanism of domestication syndrome, which can potentially be verified in further studies. An important feature of individuals of this strain is an increase of within-group morphological disparity, which is accompanied by an increase in the volume of within-group morphospace, indicating increased destabilization of development. This phenomenon is consistent with the theory of destabilizing selection by Dmirti Belyaev, who emphasized the possibility of increasing variability, the appearance of innovations and increased destabilization of development in animal selection in the direction of reducing characters of defensive behavior towards humans.

In conclusion, it should be noted that the methods of 3D geometric morphometrics in theriology are highly effective in assessing morphogenetic changes, which allows them to be used not only to solve taxonomic and phylogenetic problems, but also in studying evolutionary-ecological and ecomorphological problems associated with the analysis of morphogenesis rearrangements.

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