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Source: Wildlife Biology, 15(3) : 266-277

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/08-004

Wildl. Biol. 15: 266-277 (2009)
DOI: 10.2981/08-004

www.wildlifebiology.com

Original article

Separating wild from domestic American mink *Neovison vison* based on skull morphometrics

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Domestication can change animal traits such as skull size and shape. Given that domestic American mink *Neovison vison* may escape from farms within the native range of wild mink, we were interested in determining whether 1) skull characteristics differed between wild and domestic mink, and 2) free ranging mink in Ontario had skull features characteristic of domestic animals. Contrary to previous research, we found no effect of domestication on braincase volume or muzzle length in mink. We did, however, find that skulls of domestic mink were larger than those of wild mink and that domestic skulls had narrowed postorbital constrictions (POC). A model using both condylobasal length (CBL) and POC correctly classified the origin of 100% of male skulls and 90% of female skulls in an external data set. A POC-only model was less successful, however, correctly classifying 68% and 70% of male and female skulls, respectively. In a field application of the two-term model, only one of 109 skulls was identifed as being of farm origin. With the POC-only model, however, 12 skulls were classified as being from domestic animals. Where size differences are expected (for example, with recently escaped animals), the model should be effective for identifying domestic mink. However, the utility of CBL and POC for identifying domestic-origin or hybrid animals that have been born in the wild depends on a key uncertainty, namely, the extent to which these traits have a genetic basis.

Key words: brain size, Dollo's law, domestication, introgression, morphometrics, Neovison vison, outbreeding depression, pedomorphosis

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Received 16 January 2008, accepted 5 February 2009

Associate editor: John D.C. Linnell

The process of domestication can produce a suite of behavioural and morphological changes in animals. Some changes may be a result of intentional selection by breeders for preferred traits. For example, tamability is often a desired trait in captive animals (Hale 1969, Trut 1999). Artificial selection can also lead to unintended consequences when selected traits are genetically linked to other, unselected traits. Selection for tameness in captively bred red foxes *Vulpes vulpes* inadvertently produced a suite of morphological changes in the population, apparently due to a network of altered developmental processes governed by affected genes (Trut 1999). Domestication may also affect traits through relaxed natural or sexual selection. Domesticated animals tend to have relaxed selection for traits that increase survival or productivity in wild populations, which can lead to altered morphology, behaviour and reproduction (Price 1984, Frankham et al. 1986, Araki et al. 2007). For example, there is often a reduction in sexual size dimorphism due to relaxed sexual selection (Lynch & Hayden 1995). Finally, traits of domestic animals can also be altered from their wild counterparts by founder ef-

fects, genetic drift, or through environmental influences.

There are several morphological characteristics that are considered typical of domestic animals, and thus, may be indicative of processes leading to domestication. These include an altered body size compared to wild counterparts, a reduction in brain size, an increase in body fat, and a shortening of the facial region (O'Regan & Kitchener 2005). This generalized domestic condition has often been described as pedomorphic, resulting from increased neoteny associated with changes in the regulation of developmental processes (Price 1984, Trut 1999, O'Regan & Kitchener 2005).

The American mink *Neovison vison* is a valued furbearer endemic to North America (Lariviére 1999). The quality and varied colour of mink pelts have made mink one of the few furbearers that have been widely subjected to domestication (Joergensen 1985). Mink farmers control attributes such as reproductive efficiency and the colour, size and quality of pelts (Joergensen 1985). As a result, domesticated mink have changed morphologically and behaviourally compared to their wild counterparts. For example, domesticated mink are bred to be larger than wild mink, since larger pelts are worth more money (Joergensen 1985). They also have unintended characteristics, such as behavioural traits like aggression that are genetically linked to pelt colour (Trapesov 2000).

Several differences in skull dimensions between wild and domestic mink have also been noted. Other than larger size, the most marked difference appears to be a shortening of the muzzle in domestic mink (Bährens 1960, Pohle 1970, Lynch & Hayden 1995, Kruska & Sidorovich 2003). This may be a pedomorphic effect, or the shortened muzzle may be associated with a change in diet (Bruner et al. 2004). Kruska & Sidorovich (2003) also found an increased frontal skull height (FSH) in domestic mink. The post-orbital region of domestic mink may also be narrowed (Lynch & Hayden 1995). Finally, brain size (Kruska 1996) and braincase volume (VOL; Kruska & Sidorovich 2003) of domestic mink appear to be reduced.

In North America, there is evidence that the escape of domestic mink from farms may be common and widespread (Bowman et al. 2007). Dollo's law, which states that evolutionary trends are not reversible, suggests that domesticated traits of feral mink populations are likely retained, or at least not reversed, upon their establishment in the wild (Gould 1970, Kruska & Sidorovich 2003). Moreover, if domesticated mink hybridize with wild mink, then introgression of domestic traits such as reduced brain size might lead to outbreeding depression, if the traits are maladaptive. Indeed, reduced fitness from outbreeding depression might be an explanation for apparent declines in wild mink populations throughout Canada (Bowman et al. 2007). Concern over the status of wild mink within their native range has motivated us to test for the prevalence and effects of feral mink in Canada.

Although previous research has demonstrated likely differences in skull dimensions between domestic and wild mink (Bährens 1960, Pohle 1970, Lynch & Hayden 1995, Kruska & Sidorovich 2003), further study is required into this question because previous studies have either had relatively small sample sizes of wild mink (and therefore possible founder effects; Pohle 1970), used feral mink rather than domestic mink (Kruska & Sidorovich 2003), or used feral mink rather than wild mink (Lynch & Hayden 1995). Moreover, the mink were often from different localities and subject to different environmental influences (O'Regan & Kitchener 2005). Thus, we sought to confirm previous research suggesting morphometric variation in skull dimensions between domestic and wild mink. We also wanted to develop and test the utility of a statistical model for classifying skulls of unknown origin based on skull morphology. Given that large size is actively selected for by mink breeders, we predicted that domestic mink skulls would be larger. More importantly perhaps, we were interested in shape differences between domestic and wild animals. Shape is likely more heritable than size (Chase et al. 2002); thus, we reasoned that shape traits are more likely retained in feral animals, making shape more useful than size for classifying unknown skulls. We predicted that, compared to wild skulls, domestic mink skulls should exhibit changes that are characteristic of pedomorphosis, as has been demonstrated in other domestic animals. Pedomorphosis is the retention of juvenile-like traits in adults. Typical pedomorphic traits include reduced muzzle length and braincase volume. Based on previous mink research, we also expected to observe a reduced postorbital constriction and an increased frontal skull height in domestic mink. We developed a statistical model describing the observed differences between domestic and wild mink, and attempted to validate this model on an external data set of known-origin animals. Finally, we applied our model to a

putatively mixed population, with animals of unknown origin.

Material and methods

Skull collection, preparation, and measurement

Skulls of wild adult mink (N = 175 males and 73 females) were obtained from a collection held by the Ontario Ministry of Natural Resources. These specimens were from mink trapped by fur harvesters in central and northern Ontario during 1961-1970, in areas without known mink farms. Recent genetic analyses of mink from within this geographic area have demonstrated that the animals appear to be of wild origin (Kidd et al. 2009). Skulls of domestic mink (N = 88 males and 43 females) were acquired from a mink farm in Wheatley, Ontario, Canada, during 2006. Domestic mink were black, brown, pastel and buff in colour. Although the domestic mink were from a single farm, colours were line bred, and therefore represented distinct populations. The skulls of the domestic mink were cleaned in a dermestid beetle colony at Trent University.

Because we wanted to avoid potentially spurious effects related to growth patterns, we sampled only skulls with closed sutures, as this is indicative of the cessation of skull growth (Wiig 1985). The wild mink skulls were aged using *cementum annuli* counts or examination of skull sutures (Wiig 1985, Johnston et al. 1987). Domestic mink were of known ages; all were >6 months of age and had closed sutures. Kruska (1979) showed that skulls of juvenile mink cannot be identified morphologically after about six months of age.

Given previous findings that skull shape, and particularly VOL, of mustelids can be affected by sinus nematode *Skrjabingylus* spp. infection (Maldonado & Kirkland 1986), we sought to control for this potential effect. We assessed whether mink skulls exhibited lesions potentially associated with nematode infection, and tested for differences in skull shape and size attributable to infection. We determined that there were no differences in female skulls, but some differences in male skulls, including in VOL (Bowman & Tamlin 2007). Therefore, we excluded lesioned male mink skulls from our model development.

Based on a review of the literature related to morphometry of mink skulls (e.g. Wiig 1985, Lynch & Hayden 1995, Kruska & Sidorovich 2003), we selected 19 characteristics to measure (Table 1). We used a Mitutoyu Digimatic caliper accurate to 0.01 mm for linear measurements. Each dimension was measured three times and the median was used for subsequent analysis. VOL was estimated for each skull using the method of Eisenberg & Wilson (1978). We poured # 6 lead shot into each skull through the foramen magnum, and repeatedly tapped the skull to ensure the pellets were completely settled. We then weighed the contents on a digital Acculab pan scale (0.01 g accuracy). We repeated

Table 1. Dimensions (units in mm unless otherwise noted) measured on skulls of American mink to test for brain case size differences and skull morphology differences.

Dimension	Description	Acronym
Condylobasal length	Frontal nasal to foramen magnum	CBL
Brain basis length	Staphylion to base of foramen magnum	BBL
Palatinal length	Staphylion to front of incisors	PAL
Tooth row length	Front of incisors to last molar	TRL
Postorbital length	Postorbital process to mastoid	POL
Nasal length	Frontal nasal to postorbital process	NAL
Interorbital constriction	From side to side	IOC
Postorbital constriction	From side to side	POC
Breadth over the canini	From side to side	BRC
Mastoid breadth	From side to side	MAB
Cranial width	Greatest width of brain case	CRW
Zygomatic breadth	Greatest width of arches	ZYG
Width of the orbital constriction	From side to side	WOC
Width of the foramen magnum	From side to side	WFM
Length of the foramen magnum	From top to bottom	LFM
Caudal skull height	Basal to dorsal profile	CSH
Frontal skull height	Basal at zygomaticum to dorsal profile	FSH
Braincase height	Mastoid crest to dorsal profile	BCH
Braincase volume (cm^3)	Mass of lead pellets ÷ 6.653	VOL

this process three times for each skull and the median value was then used for analysis. Masses (M) were converted to volumes (V) using the formula V = M/6.653, where 6.653 was a constant accounting for the density of lead. Ashley L. Tamlin took all measurements of all skulls except FSH, which was measured by Jeff Bowman.

We followed the method of Lynch & Hayden (1995) to ensure repeatability of our morphometric measurements. For a stratified random sample of 12 male mink (N = 6 wild and 6 domestic), we estimated the within-individual coefficient of variation for the mean of each of the 19 measured traits (based on three measurements of each trait). We then summarized our overall measurement error as the mean of the 19 estimates from the 12 different mink (N = 228 measurements).

Sample design

Our approach was to use skulls of known wild and domestic mink for model development, and an external set of skulls of known wild and domestic mink for model validation. Skulls of wild mink from the development and validation sets were collected in areas without known mink farms, to avoid having domestic escapees potentially confounding the sample. We then sought to apply the model on a putatively mixed population, there being a high density of mink farms in the area. We carried out these steps separately for male and female skulls owing to the sexual dimorphism of mink (Wiig 1986).

We used a balanced statistical design, where sample sizes of different categories were approximately equal. We measured 180 skulls of known wild and domestic adult males, and we randomly selected 130 of these for model development (N = 65 each of domestic and wild mink). We reserved 50 for model validation (N = 23 domestic and 27 wild). The male model validation set differed from the development set only in that 50% of the validation sample consisted of skulls with lesions attributable to nematode infection, which were included to make a more robust test of the model. We had 90 skulls of known wild and domestic adult females, and we used 70 of these for model development (N = 35 of each) and reserved 20 for model validation (N = 8 domestic and 12 wild). Lesioned female skulls were included for both development and validation sets, because there are no differences in size or shape between lesioned and lesion-free female skulls (Bowman & Tamlin 2007). Finally, we sought to apply the model on a putatively mixed population. We suspected that mink occasionally escaped from farms and either became feral or perhaps even hybridized with wild mink (e.g. Bowman et al. 2007). Therefore, we applied the model to a sample of mink skulls from southern Ontario, an area that had a high density of mink farms during the period 1961-1970 when the skulls were collected (Statistics Canada catalogue 21-003). No skulls from this southern Ontario area were used in the model development or validation. We included both lesioned and non-lesioned skulls of adults (N = 83 male and 26 female skulls) in this application of the model.

Model development

All statistical analyses were carried out separately for males and females. Data from the model development sets were first visually assessed using $\log_{10}$-$\log_{10}$ bivariate scatterplots of variables compared to condylobasal length (CBL), which accounted for size variation. We used linear regressions to compare slopes of these relationships for mink of domestic and wild origin. Regression slopes and intercepts were compared using t-tests (Zar 1999) to assess differences.

Log_{10}-transformation had very little effect on relationships in the data, so for subsequent tests, we used untransformed data. Principal components analysis (PCA) was carried out to identify the variables that were most correlated with variation in skull morphology in order to reduce the dimensionality of the data sets for model development. We kept principal components with eigenvalues >1.0 (Jackson 1993), and kept the variable for each component that had the greatest loading on that component. We did not use PC scores for variables, but instead kept the measured values for selected variables. Thus, the PCA was used as a screening tool for selecting a set of uncorrelated skull dimensions.

For each sex, discriminant function analysis (DFA) using information-theoretic model selection was carried out to demonstrate the linear combinations of variables that best explained differences between mink skulls of ranch and wild origin. We used Mallow's Cp as our measure of model fit (Venables & Ripley 2002). General linear models were developed to classify mink as either domestic or wild using:

$$Y = a + b_1X_1 + \ldots b_mX_m \qquad (1),$$

where Y = a calculated value, a = constant, b = the unstandardised coefficients (from the discriminant function), and X = skull dimensions used in the

model. We were interested in both size and shape effects, although size was expected *a priori* to be different between farms and wild mink. We considered that shape effects were of particular interest because size differences might not be retained in feral animals, given that such differences may be due in part to environmental influences such as nutrition. Therefore, we selected the best models under two different scenarios: one where a size variable was included in the model (size-in), and one where size was ignored (no-size). The no-size model was different from a 'size-out' model, in that we did not remove the effects of size through regression. Instead, we simply ignored the variables highly correlated with size. This was in anticipation of a future application of the model where we hoped to be able to classify skulls based on shape characters only. For each scenario, we used the best models to classify skulls in the model development set according to domestic or wild origin to assess classification success.

Model validation

We carried out model validation for both sexes separately, and we considered the best size-in and no-size model for each. We first carried out PCAs on the model validation data and compared the components and loadings to the model development results. We then used the general linear model results from the model development analyses to generate the posterior probability (Green 1978, Brennan et al. 1986) of skulls with unknown origin being categorized as domestic or wild. The posterior probability equation is defined as:

$$P = \frac{1}{1 + (Q_2/Q_1)e^{ak+(t1+t2)/2}} \qquad (2),$$

where Q_1 = the prior probability that the skull is of wild origin, $Q_2 = 1 - Q_1$, a = a vector of skull dimensions - the X variables from (1), k = a vector of unstandardised discriminant function coefficients and constant, t1 and t2 = the mean discriminant scores (group centroids) of the domestic and wild mink. We set prior probabilities at 0.50. For each analysis, we used t-tests to compare mean posterior probability scores for the two groups (domestic and wild). We then used a probability of 0.5 as a cut point to classify skulls as domestic or wild, and used these classifications in a contingency table compared to each skull's actual origin to assess classification success. We also assessed model sensitivity (the probability of detecting true domestic mink) and specificity (the probability of detecting true wild mink).

Model application

We applied size-in and no-size models for both male and female mink to a sample of skulls from the putatively mixed population in southern Ontario. For each data set, we first carried out a PCA to once again assess components and loadings compared to the model development analysis. We then used the general linear model to generate posterior probability scores (using equation 2) for the mixed skulls. We used a probability of 0.5 as a cut point to assign skulls to either domestic or wild status.

Results

Model development

We found our skull measurements to be highly repeatable. The mean (SE) measurement error for the 19 traits measured from 12 randomly sampled mink was 0.58% (0.06).

Male and female domestic mink skulls were much larger than male and female wild mink skulls, respec-

Table 2. Size of selected skull dimensions from wild and domestic American mink collected in Ontario, Canada (N = 65 males and 35 females from each group). Dimensions included condylobasal length (CBL), frontal skull height (FSH), palatinal length (PAL), braincase volume (VOL) and postorbital constriction (POC). Except the dimension VOL, which is given in cm^3, all units are in mm.

		Wild			Domestic		
Sex	Dimension	Mean	Range	SD	Mean	Range	SD
M	CBL	64.3	59.4-69.6	2.45	73.9	67.0-78.4	2.57
F	CBL	58.1	54.7-65.6	2.16	65.2	59.0-69.8	2.65
M	FSH	16.4	15.2-17.9	0.68	19.5	17.6-20.8	0.64
F	FSH	14.7	13.5-17.1	0.71	16.9	15.6-18.4	0.75
M	PAL	26.5	28.9-31.9	1.22	30.8	34.2-37.1	1.46
F	PAL	23.5	25.7-29.1	1.20	26.0	29.7-32.2	1.36
M	VOL	8.5	7.3-10.3	0.70	10.1	8.1-11.6	0.76
F	VOL	6.7	5.8-8.2	0.58	8.0	6.4-9.6	0.63
M	POC	12.5	11.2-14.1	0.70	11.7	9.3-13.8	0.95
F	POC	11.9	10.1-12.8	0.69	11.4	9.3-12.9	0.84

Table 3. Linear regression coefficients between selected skull dimensions and condylobasal length (CBL) of wild and domestic American mink collected in Ontario, Canada (N = 65 males and 35 females from each group). Dimensions included frontal skull height (FSH), palatinal length (PAL), postorbital constriction (POC) and braincase volume ($VOL^{1/3}$). All dimensions were log_{10}-transformed. * marks pairs of coefficients that were different at $P < 0.05$.

		Wild			Domestic		
Sex	Dimension	Slope (SE)	Intercept (SE)	R^2	Slope (SE)	Intercept (SE)	R^2
M	FSH	0.79 (0.10)	-0.21 (0.17)*	0.51	0.54 (0.34)	0.29 (0.19)*	0.32
F	FSH	1.10 (0.12)*	-0.77 (0.21)*	0.73	0.69 (0.14)*	-0.02 (0.26)*	0.41
M	PAL	1.01 (0.06)	-0.36 (0.11)	0.82	1.04 (0.08)	-0.41 (0.15)	0.72
F	PAL	1.15 (0.09)	-0.62 (0.16)	0.82	1.04 (0.07)	-0.42 (0.13)	0.86
M	POC	0.29 (0.18)*	0.58 (0.33)*	0.04	-0.41 (0.29)*	1.84 (0.54)*	0.03
F	POC	0.57 (0.27)	0.06 (0.48)	0.12	0.31 (0.31)	0.50 (0.56)	0.03
M	$VOL^{1/3}$	0.46 (0.07)	-0.52 (0.12)	0.42	0.05 (0.07)	-0.54 (0.13)	0.41
F	$VOL^{1/3}$	0.58 (0.09)	-0.75 (0.16)	0.55	0.37 (0.09)	-0.37 (0.16)	0.33

tively. In fact, CBL for males was almost non-overlapping between the two groups, varying between 59.4 mm and 69.6 mm in wild mink and between 67.0 mm and 78.4 mm in domestic mink (Table 2). This size separation appeared to be greater in males than in females.

For both sexes and groups, most skull dimensions were highly correlated with skull size, which we estimated from the CBL. Of all 19 dimensions, only the postorbital constriction (POC) for three of four comparisons was not significantly related to CBL (POC was weakly related to CBL for wild females; Table 3). For simplification, we present bivariate relationships for four dimensions of particular interest to our hypotheses: POC, VOL, FSH and palatinal length (PAL). We considered PAL as indicative of the muzzle length of the skull. For the dimensions that covaried with CBL, none except FSH had regression parameters that differed significantly between domestic and wild mink (see Table 3 and Fig. 1).

PCA of the male data demonstrated two components with eigenvalues >1.0, explaining 85.05% of the total variation in the data. The most strongly loaded dimension on PC1 was CBL, which appeared to be a size-related component, whereas POC was most strongly loaded on PC2 (Table 4 and Fig. 2).

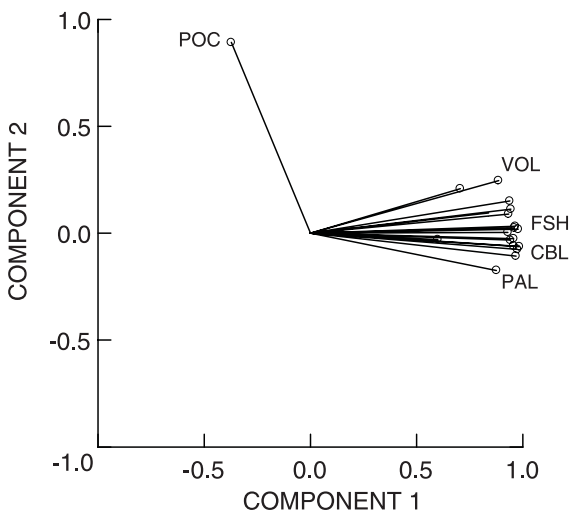

Figure 1. Bi-plot from principal components analysis (PCA) of 19 skull dimensions measured from 130 adult male American mink collected in Ontario, Canada. The five dimensions labelled are postorbital constriction (POC), braincase volume (VOL), frontal skull height (FSH), condylobasal length (CBL) and palatinal length (PAL). Axes represent loadings onto components 1 and 2. The bi-plot for female skulls was very similar.

Table 4. Loadings from principal components analysis (PCA) for 19 skull dimensions measured from 130 male and 70 female American mink. Components were included if their eigenvalues were >1.0. * denotes the most strongly loaded dimensions on each component. See Table 1 for acronyms for the different skull dimensions.

	Females		Males	
Dimension	PC1	PC2	PC1	PC2
FSH	0.949	-0.015	0.960	0.025
CBL	0.982*	0.062	0.983*	-0.063
BBL	0.936	0.113	0.942	-0.032
PAL	0.968	0.048	0.968	-0.107
TRL	0.967	0.058	0.975	-0.075
POL	0.948	0.028	0.957	-0.060
NAL	0.867	0.242	0.876	-0.174
IOC	0.933	0.021	0.944	0.113
POC	-0.009	-0.921*	-0.373	0.892*
BRC	0.972	0.068	0.957	-0.025
MAB	0.974	0.049	0.979	0.019
CRW	0.943	-0.090	0.939	0.151
JUB	0.960	0.035	0.964	0.032
WOC	0.905	-0.018	0.930	0.004
WFM	0.847	-0.075	0.839	0.094
LFM	0.373	-0.304	0.600	-0.030
CSH	0.921	-0.104	0.934	0.090
BCH	0.729	-0.115	0.706	0.208
VOL	0.901	-0.230	0.886	0.246

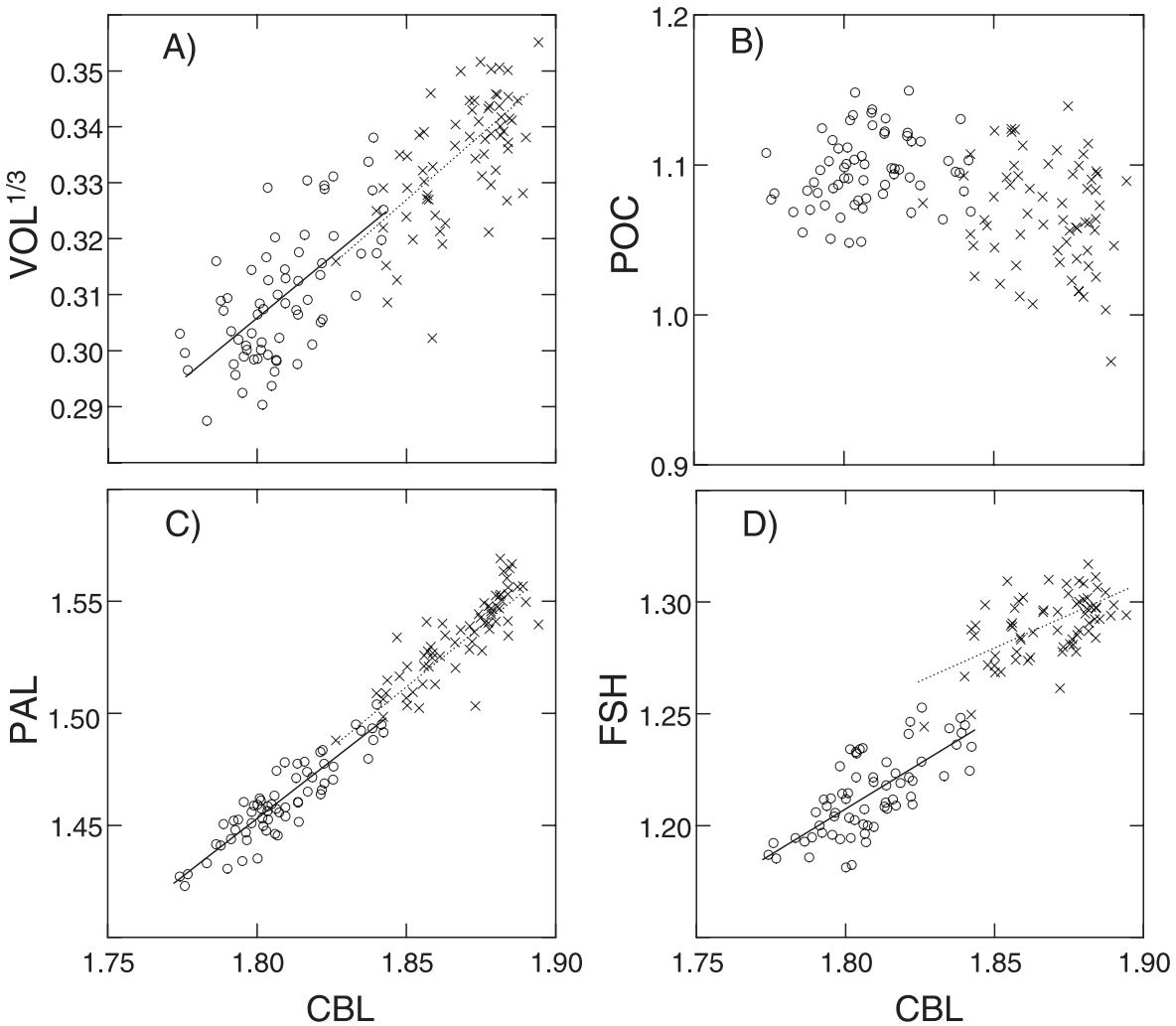

Figure 2. Linear regressions of bivariate relationships between condylobasal length (CBL) of wild (o) and domestic (×) adult male American mink (N = 65 of each group) and braincase volume ($VOL^{1/3}$; A), postorbital constriction (POC; B), palatinal length (PAL; C) and frontal skull height (FSH; D). Regression lines are not depicted for non-significant relationships ($P < 0.05$). All data are log_{10}-transformed and units are cm ($VOL^{1/3}$) or mm (all others).

For females, two components had eigenvalues > 1.0, and explained 83.3% of the total variation. Once again, the most strongly loaded dimension on PC1 was CBL, and POC was most strongly loaded on PC2 (see Table 4).

Model selection demonstrated that for both sexes, the best linear model discriminating between domestic and wild mink was one that retained both CBL and POC variables. For males, Cp for the two-term model was 7.01 (CBL-only model = 7.18, POC-only model = 27.18). For females, Cp for the two-term model was 5.44 (CBL-only model = 5.80, POC-only model 17.90). For males, discriminant function analysis using this two-term (size-in) model demon-

Table 5. Discriminant function coefficients developed from linear models depicting skull differences between wild and domestic American mink. Models were developed using 130 male and 70 female mink collected in Ontario, Canada. A 'size-in' model, including condylobasal length (CBL) as a size measure, was developed per sex. A second 'no-size' model, ignoring size was also developed, and included only postorbital constriction (POC).

Sex	Model	Discriminant function	Discriminant score: Wild (Domestic)
Male	CBL + POC	0.39 (CBL) - 0.27 (POC) - 23.43	-1.95 (1.95)
	POC	1.20 (POC) -14.46	0.48 (-0.48)
Female	CBL + POC	0.42 (CBL) - 0.44 (POC) -20.96	-1.57 (1.52)
	POC	1.30 (POC) - 14.97	0.14 (-0.14)

strated that domestic and wild mink could be separated with a linear combination of CBL and POC ($F = 244.8$, $df = 2, 127$, $P < 0.0001$, Wilks' Lambda = 0.21; Table 5). When these model development skulls were reclassified using the model, 96% were correctly assigned as domestic or wild. A discriminant function analysis (DFA) of the best no-size model (a POC-only model) also separated the two groups, but not as effectively ($F = 30.4$, $df = 1, 128$, $P < 0.0001$, Wilks' Lambda = 0.81, Classification success = 65%; see Table 5).

For females, a DFA using the two-term (size-in) model demonstrated that once again, domestic and wild mink could be separated with a linear combination of CBL and POC ($F = 81.2$, $df = 2, 66$, $P < 0.0001$, Wilks' Lambda = 0.29, Classification success = 93%; see Table 5). A DFA of the no-size model (POC-only) could not separate the two groups any better than a random model ($F = 1.4$, $df = 1, 67$, $P = 0.24$, Wilks' Lambda = 0.98, Classification success = 57%; see Table 5).

Model validation

For males in the validation data set, the first two principal components were most strongly loaded by CBL (0.986) and POC (0.944), respectively. PC1 and PC2 explained 86.1% of the total variation in the data. For females, CBL (0.990) and POC (0.947) were most strongly loaded on PC1 and PC2, respectively. These two components explained 85.5% of the variation in the data.

For both sexes we used equation 2 with each prior probability = 0.50 and coefficients from the model development to estimate the probability that a new skull was wild, for both size-in and no-size models (see Table 5). For the male size-in model, the mean (sd) probability of the wild skulls was 0.83 (0.12), and of domestic skulls was 0.15 (0.13) ($t = 19.9$, $df = 48$, $P < 0.0001$). For the male no-size model, the mean probability of the wild skulls was 0.57 (0.20), and of domestic skulls was 0.39 (0.19) ($t = 3.14$, $df = 48$, $P = 0.003$). For the female size-in model the mean probability of wild skulls was 0.79 (0.20), and of domestic skulls was 0.23 (0.20) ($t = 6.08$, $df = 18$, $P < 0.0001$). For the no-size model, the mean probability of wild skulls was 0.57 (0.21), and of domestic skulls was 0.47 (0.24) ($t = 1.0$, $df = 18$, $P = 0.332$).

Using a cut point probability of 0.50, we reclassified male and female skulls from the model validation data sets as domestic or wild. For the male size-in model, 100% of skulls were correctly classified ($\chi^2 = 50.0$, $df = 1$, $P < 0.001$). Model sensitivity and specificity were therefore 100% each. For the male no-size model, 68% of skulls were correctly classified ($\chi^2 = 6.8$, $df = 1$, $P < 0.009$). Model sensitivity and specificity were 74 and 63%, respectively. For females, 90% of skulls were correctly classified by the size-in model ($\chi^2 = 12.5$, $df = 1$, $P < 0.001$). Model sensitivity and specificity were 88 and 92%, respectively. The female no-size model classified 70% of skulls correctly ($\chi^2 = 2.8$, $df = 1$, $P = 0.094$). Sensitivity and specificity were 63 and 75%.

Model application

In general, for the putatively mixed population, skulls were more closely related in size to the wild group than to the domestic group. The mean (range; sd) CBL of males was 64.8 mm (59.6-69.6; 2.31) and of females 58.2 mm (54.1-62.2; 1.97). For the male skulls, four principal components had eigenvalues > 1.0, explaining 77.5% of the total variation. PC1 was most strongly loaded by CBL (0.943) and explained 55.6% of the total variation. PC2 was most correlated with braincase height (BCH; -0.616) and PC3 was most correlated with length of foramen magnum (LFM; 0.767). POC was most strongly loaded on PC4 (0.573). For the female skulls, there were also four principal components with eigenvalues > 1.0, explaining 81.0% of the total variation. Once again, PC1 was most strongly loaded by CBL (0.951), explaining 56.2% of the variation. PC2 and PC3 were most strongly loaded by BCH (-0.592) and nasal length (NAL) (-0.618), respectively. As with the males, POC was most strongly loaded on PC4 (0.749).

Using the size-in model for the male skulls from the mixed population, 100% of skulls were classified as wild ($N = 83$). The mean (sd) probability of these skulls being wild was 0.85 (0.10). The no-size model classified the set as 76 wild skulls (mean probability = 0.81 (0.13)) and seven domestic skulls (0.38 (0.05)) ($t = 8.8$, $df = 81$, $P < 0.001$). The size-in model classified 25 of 26 female skulls from the mixed population as wild (mean probability = 0.86 (0.11)). One skull was classified as domestic (probability = 0.42). The no-size model classified the set as 21 wild skulls (mean probability = 0.80 (0.12)) and five domestic skulls (0.41 (0.02)) ($t = 7.1$, $df = 24$, $P < 0.001$).

Discussion

In our study, domestic mink skulls were similar to skulls of wild mink in most respects, with the excep-

tions that skulls of domestic mink were larger and had reduced POC. We found no evidence of pedomorphic changes, such as reductions in muzzle length and VOL.

The greatest difference between skulls of domestic and wild mink that we observed was in size. This was not surprising because larger size is selected for in domestic mink, especially in males, as larger pelts have more commercial value (Joergensen 1985). The domestic mink in our study were larger than both those in the studies summarized by Pohle (1970) and the feral animals in the study of Kruska & Sidorovich (2003). Size was a very effective criterion for classifying the origin of mink skulls, as the size-in model classified 100% of males and 90% of females correctly in the model validation.

Size may not be that useful, however, in identifying feral, or hybrid, mink within a mixed population. Although part of the size variation may have a genetic basis (e.g. Lynch & Hayden 1995, Kharlamova et al. 2000), much of the increased size may be related to environmental influences such as nutrition (e.g. Wisely et al. 2005). Thus, we might expect size to be reduced during feralization. Supporting this contention, Kruska & Sidorovich (2003) found much less size variation between feral and wild mink skulls than we found between domestic and wild mink. It was for this reason, that we were particularly interested in shape differences between skulls of domestic and wild mink, reasoning that shape differences were likely to be more heritable (e.g. Chase et al. 2002).

Our findings concerning skull shape were not entirely consistent with results obtained in previous research. Contrary to the suggestions of Kruska & Sidorovich (2003), we found no evidence of differences in VOL between domestic and wild mink, relative to skull length. Kruska (1996) showed that brains of domestic mink were smaller than those of wild mink, and Kruska & Sidorovich (2003) suggested, based on comparing regression intercepts, that domestic mink had VOLs that were about 14% smaller than their wild counterparts. This could potentially have important implications for the fitness of feral mink compared to wild mink. We note however, that Kruska & Sidorovich (2003) did not actually report the probabilities associated with this difference in intercepts. In our case, the small differences were well within the variation in the data. Kruska & Sidorovich (2003) also combined sexes in their analysis, whereas we analyzed our data separately for each sex. We have since assessed the relationships between VOL and CBL pooled over sex and still found no difference between domestic and wild mink.

A number of authors have found a shortening of the muzzle in domestic mink (Bährens 1960, Pohle 1970, Lynch & Hayden 1995, Kruska & Sidorovich 2003). This may be a result of increased neoteny, but it has also been suggested to be associated with airorhynchy, an upward rotation of the front of the palate, resulting from a change in diet (and ultimately from relaxed selection for muzzle shape; Kruska & Sidorovich 2003). As with VOL, based on our assessment of palatinal and nasal lengths (PAL and NAL) we found no such differences between the domestic and wild mink in our study. We found PAL to be a highly repeatable measurement with very little variation (see Figs. 2 and 3), so we feel confident that we have not made a type II error.

FSH of both males and females differed between domestic and wild mink in our study, and this was consistent with the findings of Kruska & Sidorovich (2003). FSH was, however, highly correlated with size variation (see Table 4), and was therefore not a useful dimension for discriminating between domestic and wild animals.

Principal components analysis demonstrated that the most strongly loaded shape dimension, independent of size, was the post-orbital constriction (POC). Lynch & Hayden (1995) suggested that the post-orbital area is often narrowed in domesticated mustelids, and Wiig (1982) found bone resorption in this area as mink grow in order to provide room for muscle attachment. Thus, it seems that in the larger domestic mink, a narrower POC may be required to deal with the larger muscle mass of these animals. Interestingly, Kruska & Sidorovich (2003) did not identify differences in POC between feral and wild mink, suggesting that this trait, like size, may be lost through feralization. This would be true if a narrow POC does not have a genetic basis, but rather is related to nutrition through increased muscle mass.

We are uncertain why we found fewer differences in skull traits than did researchers in previous studies. We used relatively large sample sizes, and so may have avoided spurious founder effects. Also, our domestic and wild animals were geographically syntopic, with a shared climatic and environmental regime. Nevertheless, there is considerable evidence from a range of taxa that apart from effects of founders or the environment, domestication can produce pedomorphic changes in skull traits in relatively few

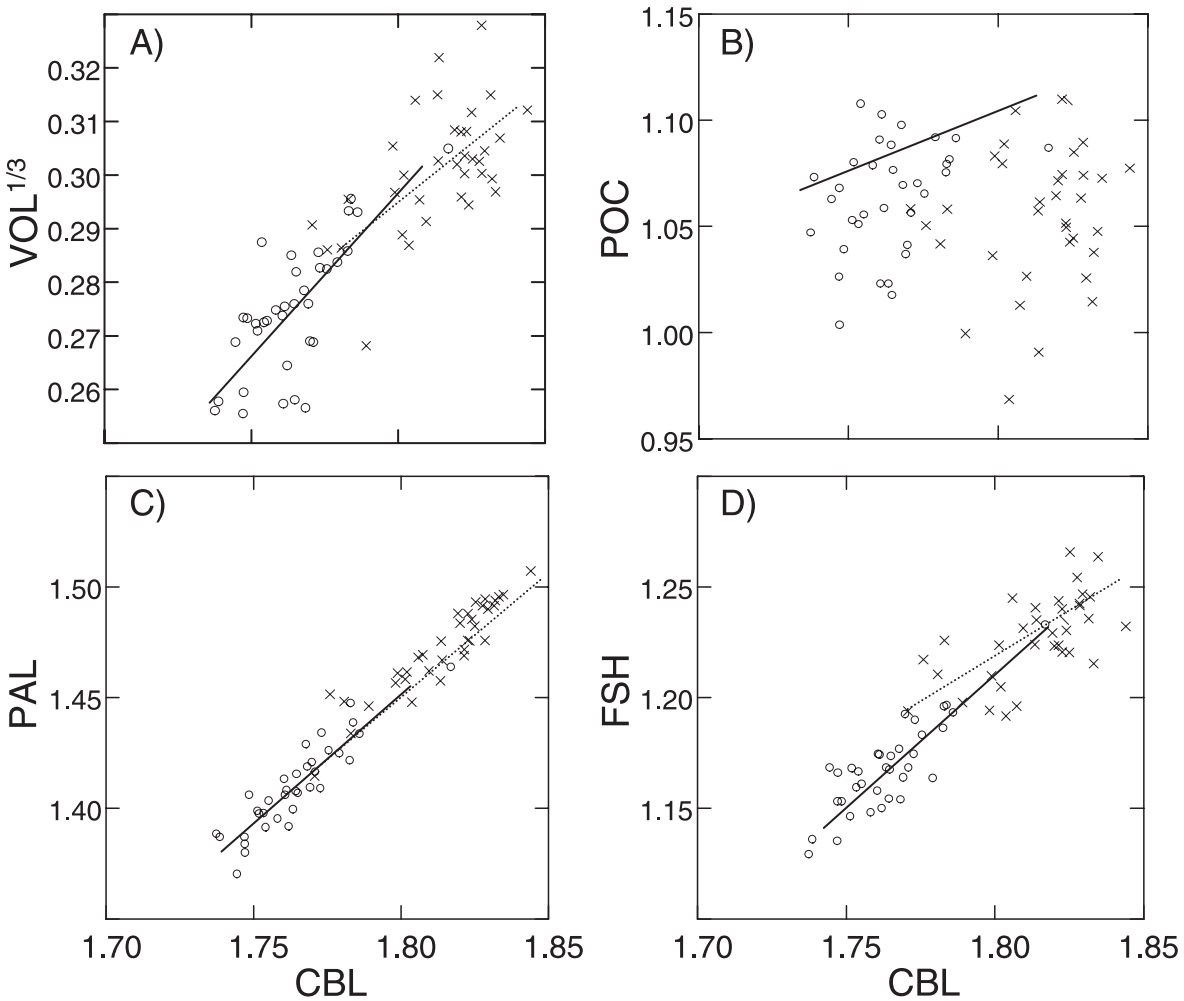

Figure 3. Linear regressions of bivariate relationships between condylobasal length (CBL) of wild (o) and domestic (×) adult female American mink (N = 35 of each group) and braincase volume ($VOL^{1/3}$; A), postorbital constriction (POC; B), palatinal length (PAL; C) and frontal skull height (FSH; D). Regression lines are not depicted for non-significant relationships ($P < 0.05$). All data are $\log_{10}$-transformed and units are cm ($VOL^{1/3}$) or mm (all others).

generations (Trut 1999, Kruska & Sidorovich 2003), and we observed no evidence of such changes. Two additional mechanisms could have limited differences between domestic and wild mink in our study. First, it is possible that some domestic mink may have been included in the wild mink model development sets, which would have served to reduce apparent differences between the groups. We attempted to preclude this with our sampling design, but cannot be completely certain that we were successful. Contemporary genetic data, however, suggest that mink from our 'wild' strata are assigned to a wild lineage (Kidd et al. 2009). Similarly, it is possible that some mink farmers occasionally have introduced wild mink as breeding stock to farms. This is legal with authorization in Ontario, but records suggest that it does not happen very often. The effect of introducing wild mink to farms would be to reduce real differences between domestic and wild animals.

Due to the dramatic size differences between domestic and wild mink in the known-origin samples used for model validation, the size-in model was highly successful. This suggests that the size-in model could be used to identify domestic mink skulls where environmental influences that might reduce size over generations are not a concern. For example, mink that were born on farms but have recently escaped would be detectable. Classification success was much lower, however, for the no-size model (68% for males and 70% for females). This suggests that if size is lost through feralization, we

have a poor ability to detect mink of domestic lineages based on skull features. This was demonstrated by our application of the model, where the putatively mixed population was much closer in size to wild mink than to domestic mink. The size-in models classified only one of 109 male and female skulls as a potential domestic animal. The no-size models classified 12 of 109 skulls as being of ranch origin. As we suspected, size became a less important characteristic in identifying domestic skulls in the mixed population. Although we have no way of assessing the success of this classification, two of these 12 skulls were noted to have come from escaped domestic mink on data forms completed during the original sample collections.

It is possible that our application of the model in southern Ontario was affected by the presence of hybrid mink. Domestic mink that escape might hybridize with wild mink. Should this happen, we would expect many trait characteristics to be intermediate, but we would also expect that variation in hybrid traits might be greater than variation in parental groups. Contrary to this expectation, the variability of traits tended to be no bigger in the putatively mixed population. For example, the coefficient of variation for CBL of male mink in the mixed population was 3.6%, compared to 3.5% in male domestic mink and 3.8% in male wild mink. Although this is not consistent with the expected pattern for the mixed population, contemporary genetic data show that there are indeed hybrid mink in the southern Ontario sample area (Kidd et al. 2009).

We have demonstrated that when size and shape are both considered, skulls of domestic mink can be reliably differentiated from wild mink skulls. Our model can be used as a quick diagnostic test for the presence of domestic skulls in a sample of potentially mixed origin. This could include museum collections or harvested animals collected by management agencies. We do not suggest that our morphometric model should replace a genetic diagnosis (e.g. Kidd et al. 2009); however, it is a quick, inexpensive alternative that could be used to initially screen samples. Moreover, in some cases (e.g. prepared museum specimens) opportunities for genetic approaches may be reduced due to poor DNA quality. Our model should be most reliable for detecting domestic mink founders within wild populations, and less so for detecting domestic-wild hybrids or backcrossed mink. At present, we do not have enough information to know whether the size and shape changes we observed in domestic skulls would likely be retained following interbreeding with wild mink. In other words, we do not know to what extent these changes are heritable. Finally, we expect that our model would perform well in other regions of the North American range of wild mink, particularly in a qualitative application. Screening a sample of skulls for relatively large CBL and small POC should identify domestic skulls, particularly where the skulls in the sample have a bimodal size distribution. Extrapolating our model coefficients should be done with caution, however, since there is considerable size variation in wild mink throughout their range. We suggest that in regions with wild mink that are much different in size, some initial model recalibration may be required.

Acknowledgements - our work was supported by the Wildlife Research and Development Section of the Ontario Ministry of Natural Resources (OMNR) and by a Natural Sciences and Engineering Research Council Discovery Grant to J. Bowman. We are grateful to former OMNR researchers, especially Audrey Fyvie (deceased) for collecting the wild mink skulls, Ed Addison and Damien Joachim for cleaning and aging the wild skulls, Jason Allen of Trent University for helping clean the domestic mink skulls, Anne Kidd for helping with domestic skull collection and lab work, and the Chesterfield mink farm for co-operating with our study. We also acknowledge Ontario's fur harvesters for providing the wild mink skulls. Finally, we thank Colin Garroway, Larissa Nituch, Albrecht Schulte-Hostedde and three anonymous reviewers for constructive comments on this article.

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