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Comparative shape analysis of pony and regular sized horse skulls

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Background. Much of the shape variation found in domesticated animals is based on allometry and heterochrony. Horses represent an excellent model to investigate patterns of size-shape variation among breeds that were intentionally bred for extreme small and large sizes.

Methods. We tested whether small horses (ponies, wither height < 148 cm) have a diverging size-shape relationship in skull shape as compared to regular sized horses (wither height > 148 cm) during ontogenetic growth. We used a dataset of 194 specimens from 26 large to regular sized horses and 13 ponies, two of which are miniature breeds – Falabella, Shetland pony. We applied three-dimensional geometric morphometrics, linear measurements, and multivariate analyses (Procrustes ANOVAs) to quantitatively examine and compare the ontogenetic trajectories between small and larger sized horse breeds with an emphasis on the miniature breeds as an extreme case of artificial selection on size. Additionally, we tested for juvenile characteristics in adult regular and miniature breeds that could resemble “paedomorphosis” – retention of juvenile characteristics in adult stage; e.g. large eyes, large braincase-to-face-relationship, and large head-to-body relationship.

Results. Allometric regression of size on shape revealed that 94% of shape variation could be explained by variation in size in all breeds. The ontogenetic trajectories of ponies and regular size breeds vary in length but not in angle. The differences in trajectory lengths result in small breeds having a similar skull shape in an older age stage than a regular size horse of the same size in a younger age stage. This pattern could cause the generally perceived “paedomorphic” appearance of small horse breeds. Miniature breeds have larger heads in relation to wither height compared to regular sized horses, a non-paedomorphic feature in horses specifically. Also rostra (faces) are longer in adult individuals than in juveniles across all kinds of breeds. This pattern can be explained by the long-face hypothesis for grazing ungulates and could possibly be caused by the mismatch of selection by humans for shorter rostra and the dentition of ruminants.

Conclusions. Ponies and miniature breed specimens do not exhibit any of the classical mammalian “paedomorphic” features (large eyes, large heads), possibly because they are herbivorous ungulates that are affected by functional and metabolic constraints put upon them by low nutrient-food. Instead ponies and miniature breeds have shorter but parallel ontogenetic growth compared to regular size breeds, resulting in adult pony skulls looking like juvenile regular breed skulls.

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Abstract

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Methods. We tested whether small horses (ponies, wither height < 148 cm) have a diverging size-shape relationship in skull shape as compared to regular sized horses (wither height > 148 cm) during ontogenetic growth. We used a dataset of 194 specimens from 26 large to regular sized horses and 13 ponies, two of which are miniature breeds – Falabella, Shetland pony. We applied three-dimensional geometric morphometrics, linear measurements, and multivariate analyses (Procrustes ANOVAs) to quantitatively examine and compare the ontogenetic trajectories between small and larger sized horse breeds with an emphasis on the miniature breeds as an extreme case of artificial selection on size. Additionally, we tested for juvenile characteristics in adult regular and miniature breeds that could resemble "paedomorphosis" – retention of juvenile characteristics in adult stage; e.g. large eyes, large braincase-to-face-relationship, and large head-to-body relationship.
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Conclusions. Ponies and miniature breed specimens do not exhibit any of the classical mammalian "paedomorphic" features (large eyes, large heads), possibly because they are herbivorous ungulates that are affected by functional and metabolic constraints put upon them by low nutrient-food. Instead ponies and miniature breeds have shorter but parallel ontogenetic growth compared to regular size breeds, resulting in adult pony skulls looking like juvenile regular breed skulls.

Introduction

Allometry, shape change associated with size change, accounts for much of newly generated shape changes (Wilson, 2018). Patterns of shape variation and allometry during ontogeny, that is the size-shape relationship during an organisms growth, have been studied in many domesticated animals such as dogs (Wayne, 1986; Morey, 1992; Goodwin, Bradshaw & Wickens, 1997; Geiger et al., 2017; Werneburg & Geiger, 2017), pigs (Hilzheimer, 1926; Evin et al., 2017), sheep (Geist, 1974), guinea pigs (Kruska & Steffen, 2013), and horses (Radinsky, 1984; Goodwin, Levine & McGreevy, 2008). Recent advances in the study of shape variation, especially through the advancement of statistical methods for the analysis of multivariate geometric morphometric data, have increased our knowledge on how shape variation arises and how variation is patterned (Adams et al., 2004; Adams, Rohlf & Slice, 2013).

In the following we investigate ontogenetic trajectories of small breeds, also known as ponies, and regular to large breeds of horses using three-dimensional geometric morphometrics (3D GM). We will highlight two special pony breeds and their position in morphospace, which have been bred for extreme small size, so-called miniature breeds, for one of which – the Falabella – so rare that we have quantified skull shape of the only complete skull specimen that is available in public museum collections. The comparative analysis of these extreme cases of miniaturisation and other breeds will give us insights into differential growth patterns in horses due to artificial selection for size.

Further, we aim to shed light on the perceived juvenile appearance of miniature breeds when compared to regular size horse breeds by assessing "paedomorphosis" in its broadest sense – the resemblance of an adult form to a juvenile of a sister group. We are aware that breeds are not individual species, for that reason we do not claim to study paedomorphosis in the strict sense. However, there are certain features in Falabella and Shetland pony adult skulls that raise the question, whether they have retained juvenile appearance. The adult Falabella skull exhibits a round braincase that can partly be seen in the other miniature breed the Shetland pony, but not in the Welsh pony (Figure 1). Generally, juvenilized phenotypic features are differences in body proportions, e.g. a larger head and shorter limbs (Gould, 1980), and differences in cranial proportions, such as larger eyes, a more prominent and bulging cranium, and a short rostrum in combination with an enlarged braincase (Gould, 1980; Wayne, 1986; Tamagnini, Meloro & Cardini, 2017; Evin et al., 2017). ~~Especially,~~ the shortening of the face portion of the skull in smaller ~~taxer~~ versus the elongation of the face portion in larger related taxa has gained some attention over the past years and has been postulated in the cranial evolutionary allometry hypothesis (CREA) (Tamagnini, Meloro & Cardini, 2017). To approach the quantification of juvenilized shape we use linear measurements derived from the 3D GM dataset for all age classes and all breeds and calculate ratios of length that reflect the typical paedomorphic traits of larger eyes, shorter face, and smaller head to body ratio in the small breeds.

Horses exhibit a large size range from 84 cm (Falabella) to 178 cm wither height (Shire). Ponies are defined by a wither height less than 148 cm (FEI, 2016). The two miniature breeds, Falabella and Shetland, formally belong to the group of ponies, collectively called small breeds hereafter. Small breeds are derived from selection on larger domesticated forms (Hendricks, 2007). Miniature breeds are defined as horse breeds with a wither height of less than 96.52 cm (38 in) ("World Class Miniature Horse Registry"). The smallest horse breed, the Falabella, originates from Argentina. The breed was first mentioned in the middle of the 19th century when very small individuals of Criollo horses were encountered in the Argentinian Pampa (Hendricks, 2007). After obtaining a few individuals and starting a breeding program with miniature horses, the name giver of the breed, Juan Falabella, added small individuals of English Thoroughbred, Criollo, and the also miniature breed Shetland pony to achieve a harmonious conformation with a wither height lower than 84 cm. The Shetland pony, which was strongly interbred with the Falabella due to its small wither height (max. 106 cm), originates from the Shetland Islands, Scotland. It is among the oldest known horse breeds and was mostly bred locally on the islands for croft works. When an act of British Parliament, however, prohibited child labour in the coalmines in 1847, the demand for these small robust ponies, as a replacement, increased drastically. Over the last century, numerous individuals have been exported, mainly being used for driving or as a first mount for children (Hendricks, 2007).

Materials & Methods

Specimens analyzed and determination of age classes

A total of 194 juvenile and adult crania were analyzed (Table S1). We examined specimens from the following collections: Museum für Naturkunde Berlin (MfN Berlin, Germany), Institut für Haustierkunde (Christian-Albrechts-Universität of Kiel, Germany), Museum für Haustierkunde

„Julius Kühn“ (University of Halle, Germany), Naturhistorisches Museum Wien (NHW Vienna, Austria), and Museo de la Plata (MLP La Plata, Argentina). The dataset includes 39 horse breeds, ranging from the smallest (Falabella) to the largest breed (Shire) (Table S1). Of the 39 breeds, 13 are considered as ponies two of which are miniature breeds: Bosnian pony (bos), Exmoor pony (exm), Falabella (fab) (miniature breed), German Riding pony (grp), Icelandic Horse (ice), Indian pony (ind), Konik (kon), Mongolian (mon), Scottish pony (scp), Shetland pony (she) (miniature), Togo pony (tog), and Welsh (wel). 26 breeds are considered normal breeds: Anglo-Norman (ano), Arab (arb), Birkenfelder (bif), Belgian Draft (blg), Clydesdale (cds), Galician Farm Horse (gbh), Grisons (Graubündner) (grb), Hannoverian (han), Hackney (hny), Holstein (hol), Hungarian (hun), Huzule (huz), Kladrubian (kdr), Kosarian (kos), Lipizzan (lpz), Nonius (nos), Norik (nor), Oldenburgian (odb), Pinzgau (piz), Polish Farm Horse (pll), Seneca Sarajevo (ses), Shire (shi), Styrian (stm), Suffolk (suf), English Thoroughbred (thb), Trakehner (trk).

Prior to analyses, each specimen was categorized into an age class from 0 to 6 using an identification key for dental eruption (Habermehl, 1975) with: 0 – dental eruption after birth, 1 – eruption of the first pair of deciduous incisors, 2 – eruption of the second pair of deciduous incisors, 3 – eruption of the third pair of deciduous incisors, 4 – eruption of the first molar, 5 – eruption of the second molar, and 6 – eruption of the third molar (Table S1). Weaning occurs around the end of age class 2 and the beginning of age class 3 (at around six months), while sexual maturity is reached at the beginning of age class 4 (at around one year) and skeletal maturity is reached in age class 5 or 6 (at around 3 to 4 years). Notably, the dataset contains only a single specimen of Falabella, to our knowledge the currently only complete skull from a museum collection to be measured.

Collection of shape data

Cranial shapes were analysed using landmark-based geometric morphometric (GMM) approaches. The crania were measured in three-dimensions (3D) using a MicroScribe ® MLX6 (Revware, Inc., Raleigh, North Carolina, USA; accuracy: 0.076 mm) and a total of 60 type I and type II landmarks (Bookstein, 1990) (Table S2, Figure S1) were collected. The dorsal and ventral sides of the crania were measured separately and the landmark datasets were subsequently combined using three reference landmarks (numbered 1, 2, and 33, Figure S1) in the

Microscribe® software MUS (Revware, Inc., Raleigh, North Carolina, USA). All subsequent analyses were conducted using R v.3.5.2 (R Core Team, 2016) in RStudio (1.2.1303) and related R packages for the analyses of geometric morphometric data (Adams et al., 2018; Dryden, 2018).

3D Geometric morphometric analyses

General Procrustes Analysis (GPA) (Rohlf & Slice, 1990) was performed on the 3D shape data to eliminate the effects of size, orientation, and scaling. GPA translates, rotates, and scales all specimens' coordinates so their centroids coincide and are scaled to unit centroid size, and the squared summed distances between matching landmarks are minimized. Due to its bilateral symmetry, only the symmetric component of the cranium was used in the subsequent analyses (Klingenberg, Barluenga & Meyer, 2002; Kolamunnage & Kent, 2003). Outliers were inspected using *geomorph:: plotOutliers* function. Centroid size for each landmark configuration was calculated using *shapes::centroid.size*.

To visualize morphospace space occupation of the age classes of miniature and regular breeds along major axes of variance, we performed a principal component analysis (PCA, *geomorph::plotTangentSpace*) using the co-variance matrix of Procrustes scores retained from the GPA. We calculated mean shapes for each age class of regular breeds as well as for the Shetland and Falabella to visualize shape differences with age.

Characterizing cranial ontogenetic shape trajectories

For subsequent analyses of ontogenetic size-shape co-variation (allometry) between and within miniature and regular size breeds we performed linear regressions (Procrustes ANOVA) of shape (Procrustes coordinates) on logarithmized centroid size, and a grouping factor, type = H/P, which denotes the group affiliation of each breed to either regular (H) or miniature (P) breed as indicated in Table S1 and raw coordinates file. To inspect the inter-specific allometric relationship between miniature and regular size breeds we used the predicted shape approach (Adams & Nistri, 2010) that plots the first principal component from a regression of predicted shape values on log centroid size. We applied a test for homogeneity of slopes (HOS) as implemented in *geomorph::advanced.procD.lm* when the interaction term of log(size) and type was significant during analysis with *geomorph:: procD.allometry* (Collyer & Adams, 2013; Collyer, Sekora & Adams, 2015; Adams & Collyer, 2016). The HOS test allowed us to determine whether miniature and regular breed ontogenetic allometries differed in length (amount of shape changes with size), slope angles (direction of shape change), or intercept.

Testing features of paedomorphosis in miniature breeds using linear measurements

Additionally, to complement the multivariate statistical analyses for differences in ontogenetic trends between small and large breeds, we aimed to test for features of "paedomorphosis" in the miniature breeds using only linear measurements. We define "paedomorphosis" loosely here, as a general resemblance of adults in miniature breeds to juveniles in all other breeds. Typical phenotypic features of paedomorphosis are differences in body proportions, e.g. a larger head and shorter limbs (Gould, 1980), and differences in cranial proportions including larger eyes, a more prominent and bulging cranium, and a short rostrum in combination with an enlarged braincase (Gould, 1980; Wayne, 1986; Tamagnini, Meloro & Cardini, 2017; Evin et al., 2017). Paedomorphism has been claimed to describe some differences among horse breeds (Budiansky, 1997; Goodwin, Levine & McGreevy, 2008), however the long-face hypothesis of grazing ungulates (Spencer, 1995), if also true for horses, could overrule any signs of paedomorphism in the rostrum. We calculated interlandmark distances (specified below) from the three-dimensional dataset in the R package *geomorph* (Adams et al., 2018) and calculated the ratios for the following three traits:

Larger eyes: To test whether miniature breeds exhibit larger orbits (eyes) than regular sized breeds relative to their respective cranial lengths, we calculated the ratio of orbit length to cranial length from measurements of the orbit diameter (LM 15 – 17, Table S2, Figure S1) and total cranial length (LM 37 – 58, Table S2, Figure S1).

Shorter rostrum: To test for rostral shortening we measured the length of and the angle between palate (LM 37 – 44, Table S2, Figure S1) and basicranium (LM 49 – 58, Table S2, Figure S1). The angle is expected to become smaller the larger the braincase and the shorter the palate becomes.

Smaller head to body ratio: We inspected the relationship of adult cranial length (LM 33 – 58) (n = 128) and average breed wither height, which we collected from the literature for a subset of 11 ponies and 18 regular breeds (breeding guidelines for each breed, Table S3). We calculated the predicted adult cranial length of the miniature breeds as derived from linear regression of adult cranial lengths from normal sized breeds (Verzani, 2014) and compared it to their actual cranial lengths. The adult cranial length to wither height ratio in relation to breed is used to investigate a possible minimal limit in cranial length in the investigated breeds.

Results

Characterization of cranial shape of miniature and normal-sized horse breeds

We calculated and visualized the mean shape for each age class from Procrustes shape data, as well as that of the adult stage of the two miniature breeds (Figure 2). A description of the different age classes (0, 3, 6) and the adult crania of the Falabella and Shetland pony are presented in Table 1. The juvenile age classes of horses are characterized by a very broad and short cranium with a bulging anterior-dorsal part of the braincase (Figure 2). During growth, the cranium elongates (rostrum stronger than the anterior part of the braincase) and the anterior-dorsal part of the cranium flattens. The orbit size decreases in relation to the complete cranium.

Cranial ontogenetic shape change

The inspection of potential outliers in the skull dataset flagged all specimens of age classes 0 and 1 as outliers regardless of group type (small or regular breed), in the sense that they fell out of the interquartile range (Figure S2). PCA (Figure 3A) reveals that the ontogenetic stages 0–6 separate in PC1-PC2 space along PC1 from adult (PC1 negative) to juvenile age stages (PC1 positive). The first PC accounts for 47.1% of the total shape variation. A gap in the shape space between age classes 0–2 and 3–6 is visible. Also, we can observe that cranial shape among early ontogenetic stages is more similar to each other the cranial shape of the adult stage, where the scatter becomes larger. When comparing the miniature breeds to the regular-sized breeds in shape space, it becomes evident that the Shetland pony specimens align with the respective age classes of other small breeds (ponies), but constitute the most ‘youthful’ cohorts of the respective stages (Figure 3A). The adult skull (age class 6) of the smallest of all horse breeds, the Falabella specimen, clusters most within age stages 3 and 4 of regular size breeds (Figure 3A, 4).

Analysis of ontogenetic allometry within and between small and regular size horse breeds

We tested for allometry and differences in allometric growth between small and regular size horses performing Procrustes ANOVAs (analyses of variance). Regression of skull shape (Procrustes coordinates) on log centroid size for the entire sample (small and regular breeds) shows a strong effect of size on shape (R^2 = 0.94, p=0.001). Adding “type” as an additional covariate yielded that mean shapes of small and regular sized breeds (F=27.0176, Z=7.878, p=0.001) as well as their allometries (F=25.725, Z=7.788, p=0.001) differ. Specifically, regular breeds show a longer trajectory (2437.331) than small breeds (1932.758) (Z=10.446, p=0.001) but not a different slope (Z=0.52, p=0.183). This result can be visually assessed in Figure 4. Given a shared common slope between the two groups, a test of LS means revealed a difference in intercept (Z=9.445, p=0.001). Generally, small breeds have attained more maturity than regular breeds at the same size, while exhibiting the same skull shape as regular breeds. The position of the adult Falabella (age class 6) skull in the allometric trend (Fig. 4) indicates that the Falabella skull shape resembles younger regular breed shapes at the same size.

Testing features of “paedomorphosis” in miniature breeds

During ontogenetic growth of regular sized horses we observe that orbits grow smaller in relation to cranial length (Figure 5 A), and the basicranium becomes shorter relative to the length of the rostrum (Figure 5 B). The angle between the basicranium and palate does not differ significantly among the different age stages (Figure 5 C). The growth pattern of Shetland ponies is similar to that of normal-sized horse breeds and do not show any signs of enlarged orbits or shortened rostra or increased brain case in the adult stage (Figure 5 A -C). The adult Falabella exhibits larger orbits (Figure 5 A) but otherwise no other juvenile features regarding the rostrum or braincase (Figure 5 A-C). The predicted cranial length of the Falabella and Shetland derived from linear regression of adult cranial lengths from normal sized breeds is 24.6 cm and 31.1 cm respectively, which contrast to their actual lengths of 35.6 cm and about 39 cm, respectively. Both actual cranial lengths fall slightly outside of the 95% prediction interval (Figure 5 D). The cranial length at the upper prediction limit raises the question whether smaller breeds are constrained to have larger crania. Examination of the cranial length to wither height ratio in relation to breed ordered by increasing maximal wither height supports that the adult miniature breeds, Shetland and Falabella, have larger crania relative to wither height than their normal-sized cognates (Figure 5 E).

Discussion

Horses show allometric cranial growth, as has been attested for most other domesticated species (Sánchez-Villagra et al., 2017), where the juvenile specimens are significantly different in cranial shape from the adult specimens. The largest shape differences in PC1-PC2 shape space in our sample can be found between the age classes 0-1 and 2-6. The difference between those two age clusters is most likely caused by the low sample size in age class 2 (n = 2) and it is likely that the ontogenetic trajectory for regular and small breeds would form a continuum if age class 2 would contain more specimens. To our knowledge, based on our examination of many museum collections, specimens of that age class are rarely available.

Ponies or small breeds and regular breeds show no differences in ontogenetic trajectory direction or angle but in trajectory length, with ponies having shorter trajectories than regular breeds. Therefore, regular breeds develop adult cranial shapes that smaller breeds will never reach during ontogenetic growth. As a result, a younger normal breed specimen and an older small breed specimen can exhibit the same skull shape. This pattern could explain why ponies look like juvenilized adult regular horses. Only the miniature breed Falabella but not the Shetland, exhibits a “paedomorphic” feature that is enlarged orbits relative to cranial length in its final age state.

We have preliminary evidence that skull shape differences among pony breeds arise prenatally. We compared the ontogenetic trajectories of Shetland Ponies as a miniature breed and the Welsh as a regular pony (Figure S3, not presented in main text due to small sample size). Apart from dogs (Werneburg & Geiger, 2017), this pattern has also been hypothesized in pigs (Evin et al., 2017), and needs to be further investigated in horses including more breeds.

We investigated whether ponies, when compared to regular size breeds, represent a case of craniofacial evolutionary allometry (CREA) (Cardini & Polly, 2013; Cardini et al., 2015; Tamagnini, Meloro & Cardini, 2017). CREA predicts that larger forms are long-faced and smaller forms short-faced as a sign of paedomorphism. As an approximation for braincase-to-face relationship we calculated the ratio of basicranium-to-palate lengths for miniature and regular size horses. We found no signs of CREA. In contrast, we found that our results are in accordance with the long-face hypothesis for grazing ungulates (Spencer, 1995). The long-face hypothesis does not offer a definite explanation why longer faces are observed in smaller forms of grazing ungulates. In the case of the miniaturized horses, we propose that this could be due to constraints in tooth morphology and the feeding style of grazing. Among veterinarians, it is commonly known that miniature horse breeds have a higher requirement for veterinary dentist procedures, due to their almost regular horse-sized teeth (Wilson, 2012). The same health-related problem has been shown for pet rabbits, which experience a rostral shortening through domestication without a change in dentition (Böhmer & Böhmer, 2017). These functional constraints have been investigated also in humans showing that miniature forms tend to have relatively larger teeth than regular sized forms (Shea & Gomez, 1988). Since horses feed on a very nutrient poor diet, they are in need of a highly specialized feeding apparatus to ensure the best energy recovery possible. A strong shortening of the rostrum, as can be found in some dog and pig breeds (Geiger & Haussman, 2016; Evin et al., 2017), is most likely possible due to their energy rich diet (carnivore/omnivore) that can be exploited with fewer, smaller, or differently placed teeth. In cows, which also feed on nutrient poor grass, one case of rostral shortening is known: the Niata breed (Veitschegger et al., 2018); this was likely possible due to the more efficient uptake of nutrients through rumination. To our knowledge, there is not a single case of rostral shortening in any herbivore, non-ruminant mammal species, neither by natural not artificial selection.

Large heads and increased head-to-body ratio has been shown to be a paedomorphic feature in other domesticated species, namely dogs and chicken (Alberch et al., 1979; Gould, 1980; Wayne, 2001), but does not associate with paedomorphosis in horses as new born foals have shorter rostra than adult individuals because teeth development drives rostral lengthening; as well as foals having relatively longer legs (Habermehl, 1975; van Heel et al., 2006; Goodwin, Levine & McGreevy, 2008) (Table 1). The long legs in horses are a necessity for surviving, since the new born foals are very precocial and need to keep up with the herd from day one. Our study did not compare the actual limb to head length ratio which has been proposed as a sign for paedomorphism (Goodwin, Levine & McGreevy, 2007, 2008), but used wither height as a proxy for size. We found that the miniature breeds have larger heads relative to body size (approximated by wither height) when compared to regular size breeds. So adult miniature breeds do not exhibit the juvenile state of a regular size horse in respect to head-to-body size ratio.

Regarding the adult Falabella skull and the case of the Falabella in general, we could not support our first subjective impression that the Falabella must be a “paedomorphic” horse.

However, this impression probably derived from the very round anterior-dorsal part of the braincase, whose geometric morphometric quantification by using true landmarks eluded us due to the lack of sutures in that portion of the cranium, but the curvature can be seen in the photograph of the Falabella cranium (Figure 3A). Additionally, the Falabella does exhibit a less downward curved rostrum than adults of normal-sized breeds (Figure 1). For a better assessment of a rounded cranium roof, future investigations are advised to use semi-landmarks or polygons (MacLeod, 2013; Collyer, Sekora & Adams, 2015).

Conclusions

We investigated patterns of allometry during ontogeny in horses as a case of directional artificial selection for extreme size differences in domesticated horses. Wither heights range from 84 cm in the Falabella to 178 cm in the Shire horse. We looked at allometric trends between ponies and regular size horses and investigated typical patterns of "paedomorphosis", defined as juvenile appearance in adult stage, with an emphasis on miniature horses as an extreme case of size selection. We found that ponies and regular size breeds have shifted ontogenetic trajectories that vary in length but not in direction or angle, with the consequence that small breeds exhibit similar skull shapes at older age stages than regular size breeds at younger age stages. This pattern is a potential source of the perceived juvenilized skull shape of ponies, additionally to features of the postcranial architecture, namely general small size or short limbs, or behavioral aspects as has been show in dogs (Hare & Woods, 2013), such as the use of body language (Goodwin, Bradshaw & Wickens, 1997) or facial expressions (Waller et al., 2013).

Other than the overall shape development of small breeds halting earlier than of regular size breeds we find no other evidence of "paedomorphic" features, as enlarged orbits, shorter faces, or increased head-to-body ratio, as it is the case in dogs and pigs, except for the adult Falabella skull. Miniature breeds have increased skull-to-body ratio when compared to regular size breeds. This could be due to the very essence of horses, that is being a grazing ungulate as postulated in the long-face hypothesis (Spencer, 1995). We propose functional and metabolic constraints rather than flight responses as a potential driver of this pattern.

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Table 1(on next page)

Table 1. Description of morphological differences for three age classes of medium and large breeds (0,3,6) and age class 6 for both miniature breeds (Falabella, Shetland) for the studied sample by module (for a detailed sample composition see Table S1).

Table 1: Description of morphological differences for three age classes of medium and large breeds (0,3,6) and age class 6 for both miniature breeds (Falabella, Shetland) for the studied sample by module (for a detailed sample composition see Table S1).

	Medium and large breeds			**Miniature breeds**	
Module	**Age class 0**	**Age class 3**	**Age class 6**	**Falabella (age class 6)**	**Shetland (age class 6)**
Anterior-oral-nasal	Short, very round, and narrow premaxillare; no incisors; maxillare in diastema much narrower as premaxillare; nasale and diastema are straight; diastema is very short	Elongated and broader; Third pair of incisors erupted; elongated diastema; nasale is straight or curved depending on breed; maxillare in diastema almost as broad as premaxillare	Elongated and broader; premaxilla-maxilla suture closed; elongated diastema; nasale is straight or curved depending on breed; maxillare in diastema almost as broad as premaxillare	Short, round, and broad premaxillare; maxillare in diastema almost as broad as premaxillare; all incisors fully erupted; nasale is concave	Elongated and broader; premaxilla-maxilla suture closed; elongated diastema; nasale is slightly convex; maxillare in diastema almost as broad as premaxillare
Orbital	Round or egg-shaped depending on individual; large compared to skull length; post-orbital margin is thin	Round or egg-shaped depending on individual; medium compared to skull length; postorbital margin has thickened	Round or egg-shaped depending on individual; small compared to skull length; postorbital margin is thick	Round or egg-shaped depending on individual; medium compared to skull length; postorbital margin is thick	Round or egg-shaped depending on individual; small compared to skull length; postorbital margin is thickened
Zygomatic-pterygoid	Frontal-zygomatic and temporal-zygomatic suture open; facial crest, zygomatic, and temporal form a straight line in lateral view	Frontal-zygomatic and temporal-zygomatic suture started to close; facial crest, zygomatic, and temporal form a straight line in lateral view	Frontal-zygomatic and temporal-zygomatic suture closed; facial crest and zygomatic form a straight line in lateral view; temporal is curved from lateral view	Frontal-zygomatic and temporal-zygomatic suture started closing; facial crest, zygomatic, and temporal form a curved line in lateral view	Frontal-zygomatic and temporal-zygomatic suture closed; facial crest and zygomatic form a straight line in lateral view; temporal is slightly curved from lateral view
Cranial base	Round; occipital condyle and paracondylar process have a similar length; basisphenoid-presphenoid and basisphenoid-occipital suture open; basillar part of the occipital is broad	Elongated and distinct; basisphenoid-presphenoid started to close and basisphenoid-occipital suture open; basillar part of the occipital is elongated; paracondylar process is slightly longer than paracondylar process	Elongated and very distinct; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basillar part of the occipital is elongated; paracondylar process is much longer than paracondylar process	Short and broad; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basillar part of the occipital is broad; paracondylar process is longer than paracondylar process	Elongated and very distinct; basisphenoid-presphenoid and basisphenoid-occipital suture closed; basillar part of the occipital is elongated; paracondylar process is much longer than paracondylar process

Cranial vault	Occipital is not fused to any other bone; very round; frontal-parietal-occipital doming; occipital crest very small	Frontal-parietal doming; occipital elongated; occipital crest more pronounced; occipital started fusing to surrounding bones	Frontal-parietal-occipital flattened; occipital elongated; occipital crest very pronounced; occipital mostly fused with surrounding bones	Frontal-parietal-occipital doming; occipital elongated; occipital crest very pronounced; occipital mostly fused with surrounding bones	Frontal-parietal-occipital doming; occipital elongated; occipital crest very pronounced; occipital mostly fused with surrounding bones
Age classification	First post-natal stage, before the eruption of the first pair of incisors, up to 1 week old	Time after the eruption of the third pair of incisors until the eruption of the first molar, six month to one year, before sexual maturity; weaning is around 6 month of age	Last age stage after the eruption of the third molar, from 4 years on, skeletal maturity	Adult, age stage 6	Adult, age stage 6

Figure 1

Figure 1: Cranial shape comparison among two miniature breeds and regular sized breeds through ontogeny.

Examples of different cranial shapes during ontogeny from lateral view if available for each age class (0-6) for Falabella and Shetland (miniature breeds) and Welsh (pony); each stage is represented by a different individual and all crania are scaled to the same length for comparison. Photographs by Laura Heck.


B
C
Age class
0
1
2
3
4
5
6
A
Falabella
Shetland Pony
Welsh

Figure 2

Figure 2. Cranial mean shapes for adult Falabella and Shetland, and for each analyzed age class of regular sized horses.

Cranial shapes in lateral and dorsal view for A) the average shape of each age class (0-6) of all specimens belonging to a regular sized breed (for detailed sample composition see Table S1), B) the Falabella and C) the average shape of age class 6 of Shetland ponies; all crania are scaled to the same length for better comparison.

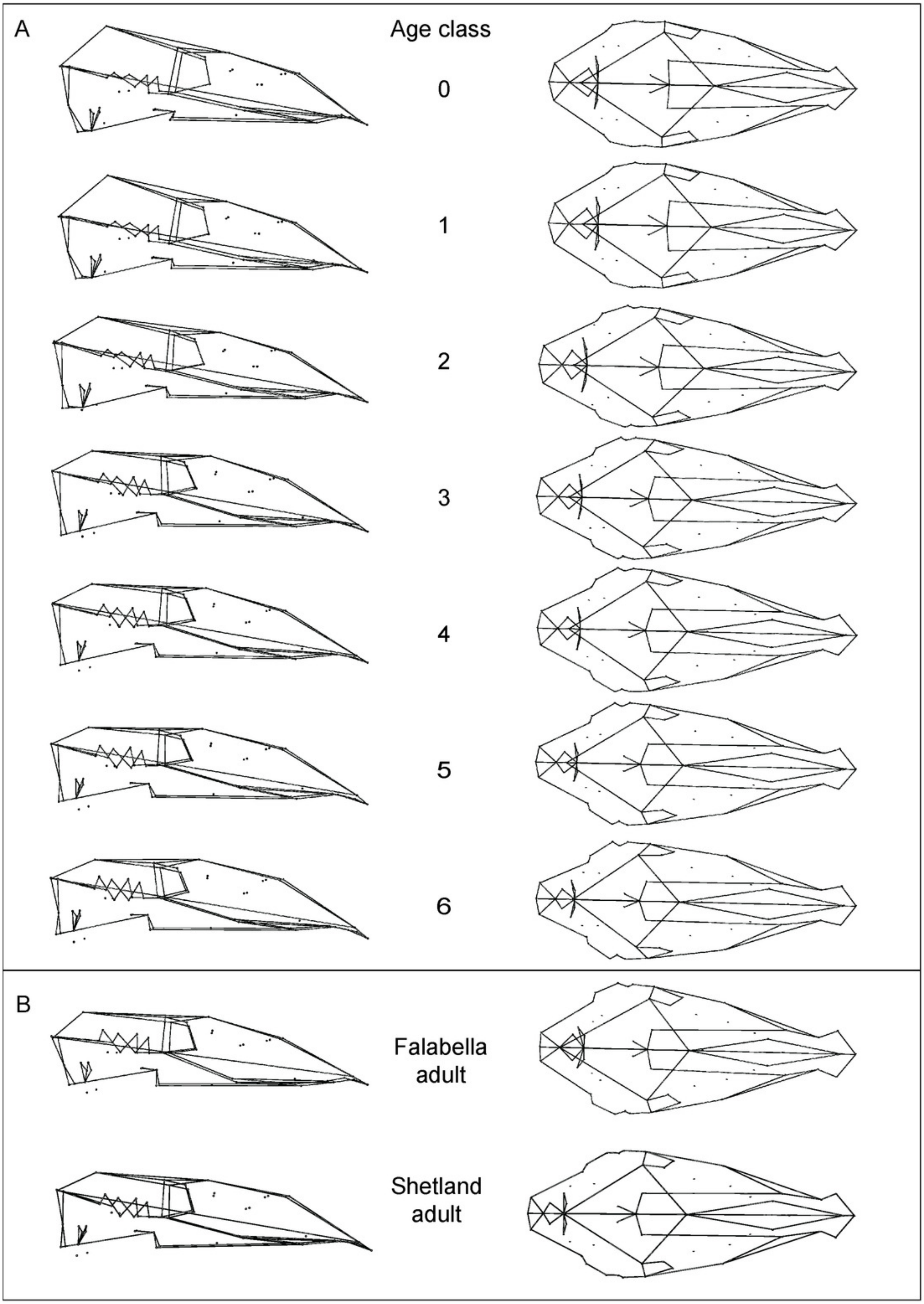

A
Age class
0
1
2
3
4
5
6
B
Falabella adult
Shetland adult

Figure 3

Figure 3. Principal component analysis of 194 specimens of 37 regular size and small breeds and two miniature breeds.

A) PC1-PC2 scatterplot shows ontogenetic trajectory for all analyzed horse breeds (see Table S1 for details). Miniature breeds, breeds of extreme small size are highlighted. B) Lateral and dorsal views of the cranium show the shape changes along PC1, adult shape in grey and juvenile shape in black. Colors represent age classes.

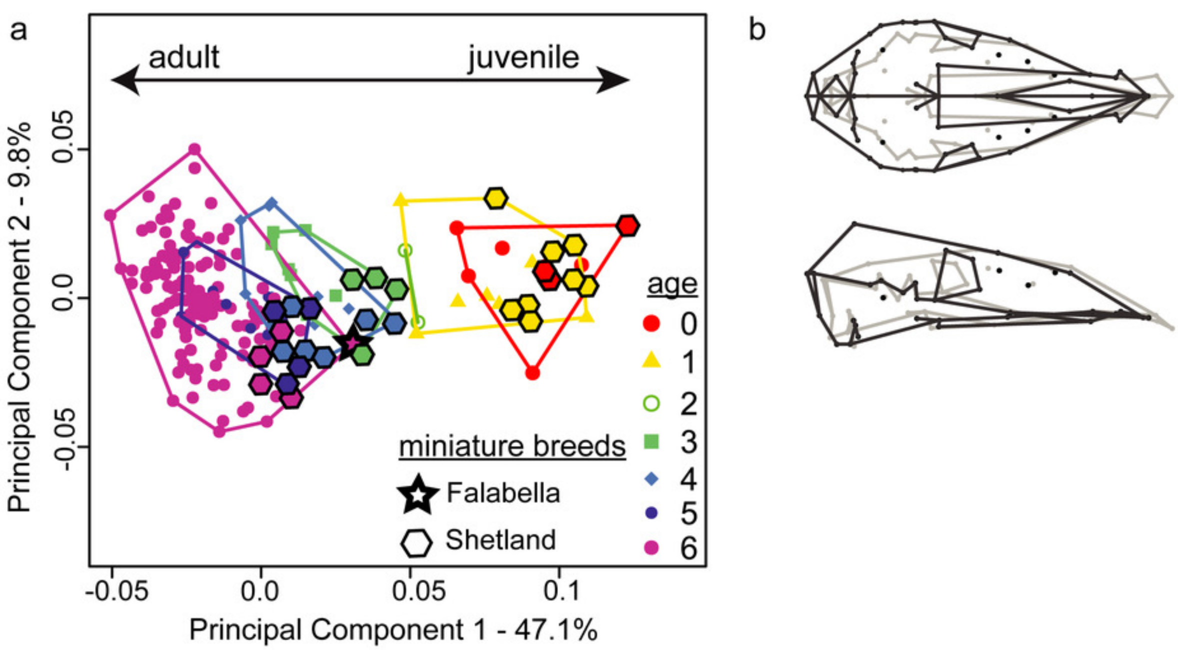


Figure 4

Figure 4. Allometric trend in small and regular size horse breed skull shapes.

Predicted shape values from regression of shape on log centroid size. Small breeds are shown in stars, regular breeds in open squares. The position of adult Falabella skull is highlighted. Colours correspond to age classes.

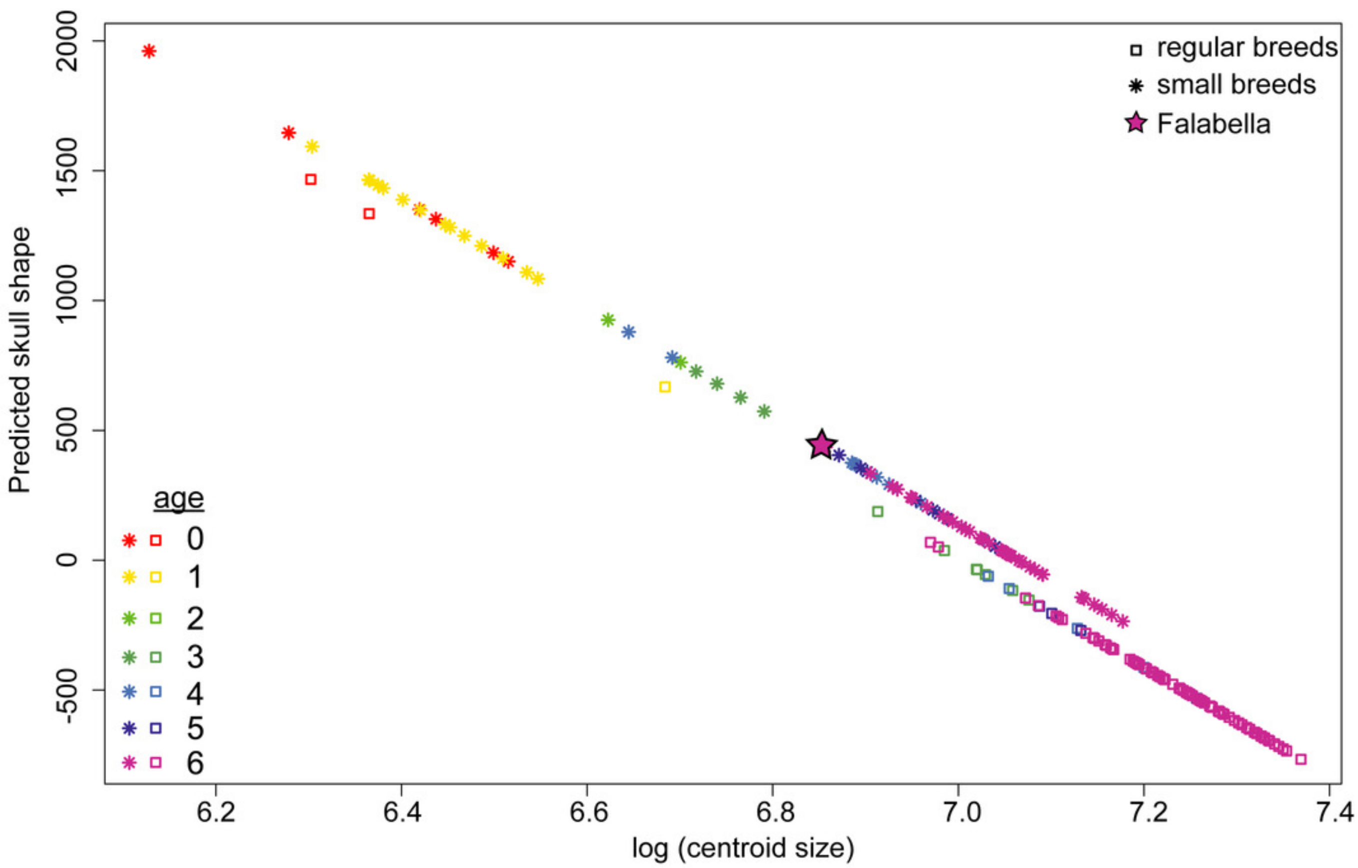
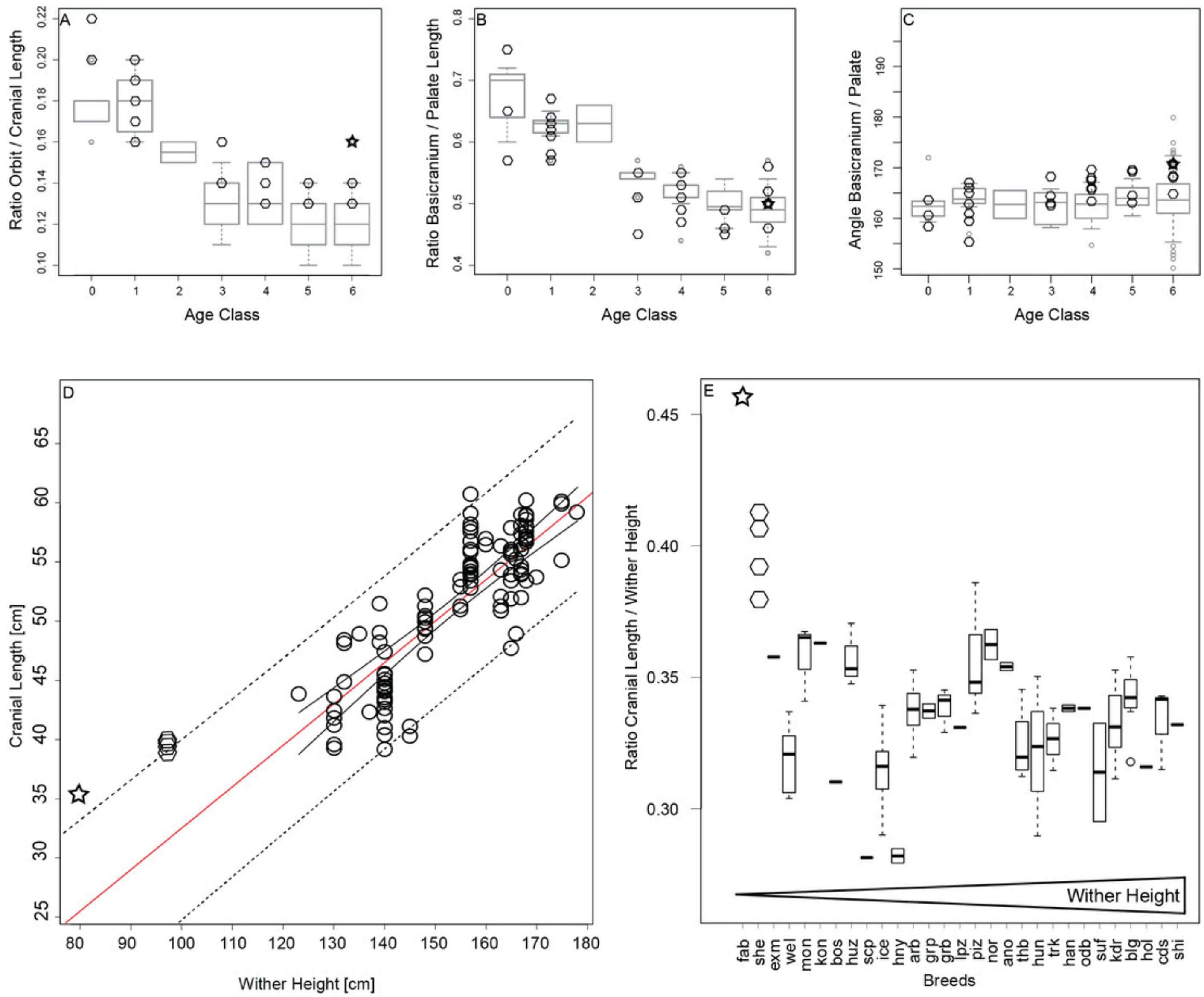


Figure 5

Figure 5. Testing for “paedomorphic” features.

(A-C) Orbit to cranial length ratio, basciranium to palate length ratio, and angle between basicranium and palate, per age category, normal-sized breeds as boxplots in grey, with Shetland ponies (diamonds) and Falabella (star) superimposed in black. (D) Adult cranial length in relation to maximal wither height with 95% confidence interval (solid line) and prediction interval (dotted line), and regression line (red). Regression line and 95% prediction curves were extended to smaller wither heights to accommodate the cranial length of the miniature breeds in the same plot. (E) Adult cranial length to wither height ratios were plotted against breed in ascending order from the smallest breed, the Falabella, to the largest breed, the Shire horse. Abbreviations for the breeds: fab: Falabella; she: Shetland pony; exm: Exmoor pony; wel: Welsch, mon: Mongolian; kon: Konik; bos: Bosnian pony; huz: Huzule; scp: Scottish pony; ice: Icelandic Horse; hny: Hackney; arb: Arab; grp: German Riding pony; grb: Grisons; lpz: Lipizzan; piz: Pinzgau; nor: Norik; ano:Anglo-Norman; thb: English Thoroughbred; hun: Hungarian; trk: Trakehner; han: Hannoverian, odb: Oldenburgian; suf: Suffolk; kdr: Kladrubian; blg: Belgian Draft; hol: Holstein; cds: Clydesdale; shi: Shire; for the ten breeds that were included in 3D GM but not linear analyses, no information on average wither height could be found.


A
Ratio Orbit / Cranial Length
0.10 0.12 0.14 0.16 0.18 0.20 0.22
0 1 2 3 4 5 6
Age Class
B
Ratio Basicranium / Palate Length
0.4 0.5 0.6 0.7 0.8
0 1 2 3 4 5 6
Age Class
C
Angle Basicranium / Palate
150 160 170 180 190
0 1 2 3 4 5 6
Age Class
D
Cranial Length [cm]
25 30 35 40 45 50 55 60 65
80 90 100 110 120 130 140 150 160 170 180
Wither Height [cm]
E
Ratio Cranial Length / Wither Height
0.30 0.35 0.40 0.45
Wither Height
fab she exm wel mon kon bos huz scp ice hny arb grp grb lpz piz nor ano thb hun trk han odb suf kdr blg hol cds shi
Breeds