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Morphological integration in the appendicular skeleton of two domestic taxa: the horse and donkey

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Organisms are organized into suites of anatomical structures that typically covary when developmentally or functionally related, and this morphological integration plays a determinant role in evolutionary processes. Artificial selection on domestic species causes strong morphological changes over short time spans, frequently resulting in a wide and exaggerated phenotypic diversity. This raises the question of whether integration constrains the morphological diversification of domestic species and how natural and artificial selection may impact integration patterns. Here, we study the morphological integration in the appendicular skeleton of domestic horses and donkeys, using three-dimensional geometric morphometrics on 75 skeletons. Our results indicate that a strong integration is inherited from developmental mechanisms which interact with functional factors. This strong integration reveals a specialization in the locomotion of domestic equids, partly for running abilities. We show that the integration is stronger in horses than in donkeys, probably because of a greater degree of specialization and predictability of their locomotion. Thus, the constraints imposed by integration are weak enough to allow important morphological changes and the phenotypic diversification of domestic species.

1. Introduction

An understanding of evolutionary mechanisms requires a good grasp of the factors that constrain or drive phenotypic diversity. Indeed, different processes are known to affect the propensity and direction of variability and may influence the variation that is available for selection to act upon [1,2]. Notably, phenotypic variation of morphological structures is largely influenced by interactions among their component parts. The tendency of different traits of an organism to covary is defined as morphological integration [3–5]. It is produced by the sharing of biological processes such as the same developmental origin or the implementation of the same function [3,4,6–11]. This biological organization of organisms can be conserved and translated into a pattern of evolutionary integration [12]. In tetrapods, the fore- and hind limbs are serially homologous structures, produced by the duplication and expression of the same developmental programme in multiple locations and times [13]. This shared developmental origin of fore- and hind limbs is hypothesized to have produced a strong integration between serially homologous elements [9,14,15]. The covariation between serially homologous bones, an ancestral condition, can be modified in response to functional requirements [1,16] and can decrease with the functional divergence between the fore- and hind limbs due to specialization of one of the limb pairs. Concomitantly, integration can be stronger across within-limb

elements suggesting the functional importance of the module in the behaviour of the animal [2,5,6,15,17–22]. Furthermore, serially homologous bones do not functionally correspond to each other due to the reorganization in the skeleton of therian mammals [20,21]. Indeed, the reduction of the coracoid, resulting in the mobility of the scapula in the parasagittal plane, and the subdivision of the autopodial element at the ankle joint [23,24] caused a dissociation between serially homologous and functionally equivalent bones. This overlap of functional and developmental units in mammalian limb bones produces a complex structure of covariation between bones [20]. A better understanding of the factors producing morphological integration can be obtained by studying the patterns of covariation in order to assess the mechanisms that drive or constrain phenotypic diversification.

Most studies on morphological integration have focused on natural populations and have described macroevolutionary patterns and trends. On the contrary, less attention has been paid to domesticated species which are, however, particularly suitable to study microevolutionary processes [25]. Indeed, artificial selection on domestic species can generate large morphological change in a very short time. Because any change on an element produces change on the elements with which it covaries, strong morphological integration could constrain the variation and thus, also limit the variation available for selection to act upon. This raises the question of how integration may constrain the morphological diversification of domestic species. Here, we focus on the morphological integration of domestic taxa that are under strong artificial selection: the horse (*Equus caballus*) and the donkey (*Equus asinus*). Both are economically important for human societies and are used for transport, agriculture, or equestrian leisure [26]. Since the eighteenth century, the domestic horse has experienced strong artificial pressures for performance or to develop specific phenotypes [27–30]. Its selection has resulted in a large range of breeds that were established recently or are still being established [31]. On the other hand, donkey breeds typically find their origin in regional types and were often selected for polyvalent functional tasks when not being bred for mules [26] and can, therefore, be considered as having been subjected to a lower-intensity artificial selection compared with horses.

No study to date has explored the integration patterns in the skeleton of domestic equids. We predict that, as the limbs of equids are exclusively involved in locomotion, the absence of a functional divergence between fore- and hind limbs in these taxa will produce a high degree of covariation between them, and probably strong global integration. On the other hand, the intense artificial selection on these organisms is ongoing and consequently, we hypothesize that this requires morphological structures that can vary easily [1]. This raises the question of whether artificial selection produces a reorganization of the integration patterns in order to allow morphological traits to vary [25]. This study investigates how developmental and functional mechanisms impact the covariation structure in equids, allowing us to evaluate whether organisms subjected to artificial selection present similar patterns of integration compared to wild quadrupeds. Furthermore, the comparison between the results obtained for horses and donkeys could reveal the impact of functional differences on covariation structure due to the differential degree of natural or artificial selection on locomotor specialization between these two species.

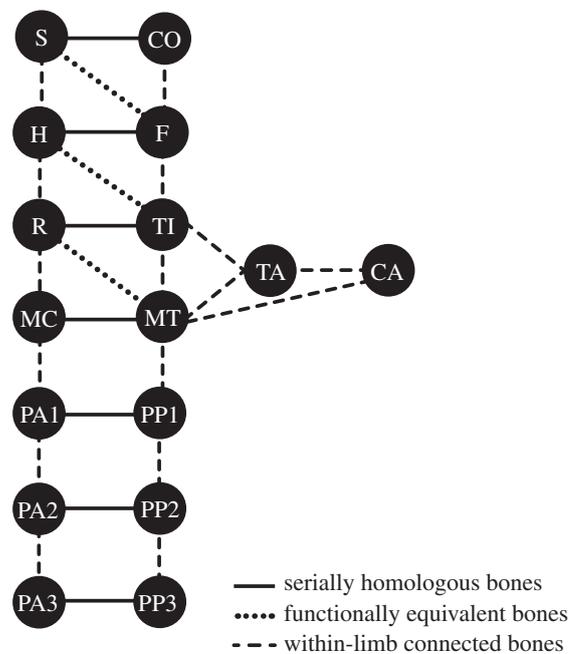


Figure 1. Graphical models of hypotheses of morphological integration in the appendicular skeleton of equids between serially homologous bones (solid lines), functionally equivalent bones (dotted lines), and within-limb connected bones (dashed lines). S, scapula; H, humerus; R, radio-ulna; MC, metacarpal bone; PA1, proximal anterior phalanx; PA2, middle anterior phalanx; PA3, distal anterior phalanx; CO, coxal bone; F, femur; TI, tibia; TA, talus; CA, calcaneus; MT, metatarsal bone; PP1, proximal posterior phalanx; PP2, middle posterior phalanx; PP3, distal posterior phalanx.

2. Material and methods

(a) Material

The dataset includes the complete or partial skeletons of 75 individuals housed in the collections of several European institutions (see electronic supplementary material, S1). The sample was selected to be representative of a large morphological diversity. It is composed of 41 domestic horses (*E. caballus*), including the broadest possible diversity in terms of breeds, and 34 domestic donkeys (*E. asinus*). The sample consists of both males and females. Only adult specimens with fully fused epiphyses were used.

(b) Acquisition of data and three-dimensional geometric morphometrics

For each complete equid skeleton, the three-dimensional coordinates of anatomical landmarks were registered on 16 bones (scapula, humerus, radio-ulna, metacarpal bone, coxal bone, femur, tibia, calcaneus, talus, metatarsal bone, proximal, middle, distal anterior phalanges and proximal, middle, distal posterior phalanges) using a Microscribe 3D digitizer. The landmarks were defined according to the protocol of Hanot *et al.* [32], but some landmarks were removed from the analyses (see electronic supplementary material, S2). Coordinates were aligned by a generalized Procrustes analysis (GPA). To quantify the shape covariation, partial least-squares coefficient (r_{PLS}) [33] and covariance ratios (CR) [34] were used jointly, as recommended by Adams [34]. Details concerning the statistical analyses can be found in electronic supplementary material S3. The degree of covariation was measured between serially homologous bones, between functionally equivalent bones, and between within-limb connected bones (figure 1).

Because size affects the different components of an organism and, thus, contributes to producing integration [10], the influence of allometry (the influence of size on shape) on integration patterns was assessed. Procrustes ANOVA with permutation procedures

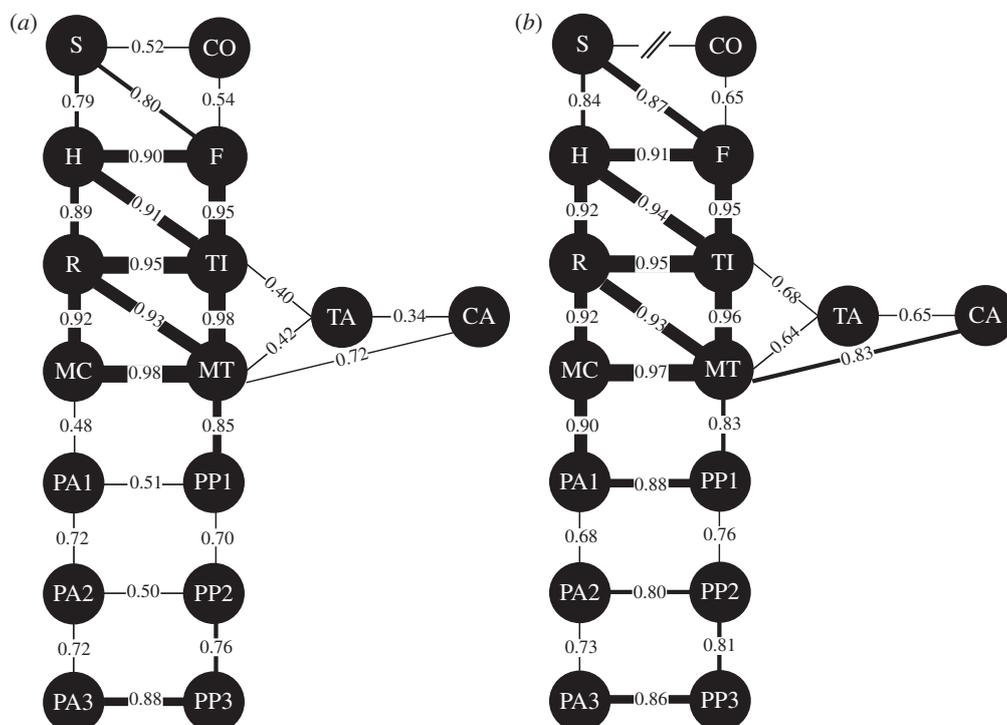


Figure 2. Graphical models of the CR and PLS coefficients obtained on the appendicular bones for the total sample of domestic equids. The line thickness is proportional to the coefficient values (the boldest lines corresponding to the strongest intensity of covariation). The absence of covariation (non-significant PLS result) is represented by a double slash '//'. S, scapula; H, humerus; R, radio-ulna; MC, metacarpal bone; PA1, proximal anterior phalanx; PA2, middle anterior phalanx; PA3, distal anterior phalanx; CO, coxal bone; F, femur; TI, tibia; TA, talus; CA, calcaneus; MT, metatarsal bone; PP1, proximal posterior phalanx; PP2, middle posterior phalanx; PP3, distal posterior phalanx. (a) CR values, (b) PLS values.

were performed to quantify allometry and allometric curves (multivariate regressions between the shape variables and \log_{10} centroid size) were plotted using the function `procD.allometry` from the R package 'geomorph' [35].

3. Results

(a) Allometry

Allometry is significant for most of the bones. However, the results reveal that the allometric slopes between the groups of horse breeds are not parallel, except for the calcaneus ($p = 0.08$) and the distal posterior phalanx ($p = 0.45$). This absence of homogeneity of slopes prevents us from calculating common allometric directions [36] by multivariate regression [37]. Thus, it is impossible to estimate the allometry-free residuals in order to observe the allometry-free covariation patterns [10].

(b) Morphological integration in domestic equids

It should be noted that the analyses performed on the total sample, by including all the specimens of both horses and donkeys, result in mixing the effects of intra- and interspecific covariations which may be produced by different processes. Nevertheless, the plots of the PLS analyses (see electronic supplementary material, S4) show that the two species are clearly separated along the first covariation axis of all the studied couples of bones. This indicates that the covariation analyses performed here mainly contain interspecific information which can be considered as more likely reflecting the impact of functional adaptations [20].

(i) Serially homologous bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation

within bones is higher than between bones for all the serially homologous elements (figure 2a). CR and PLS values indicate that there is strong covariation (PLS > 0.90) between serially homologous long bones (humerus/femur, radio-ulna/tibia, and metacarpal/metatarsal) with decreasing values from the most proximal to the more distal pairs of bones (figure 2). The coefficients also indicate that there is significant covariation between the serially homologous phalanges but the lower values (PLS ≤ 0.80) reveal that they are more weakly integrated. Moreover, there is no significant covariation between the scapula and coxal bone, according to the PLS coefficient, which is concomitant with the low CR value.

(ii) Functionally equivalent bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the functionally equivalent elements (figure 2a). All the PLS-associated permutation tests are significant, indicating significant covariation between the functionally equivalent elements (figure 2b). CR and PLS coefficients indicate that there is strong covariation between the functionally equivalent bones humerus/tibia and radio-ulna/metatarsal (PLS > 0.90).

(iii) Within-limb connected bones

All the CR-associated permutation tests are significant, with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the within-limb connected elements (figure 2a). All the PLS-associated permutation tests are significant, indicating significant covariation between the within-limb connected elements (figure 2b). CR and PLS coefficients indicate that there is stronger covariation (PLS > 0.90) between long bones (humerus/radio-ulna, radio-ulna/metacarpal, femur/tibia, and tibia/metatarsal)

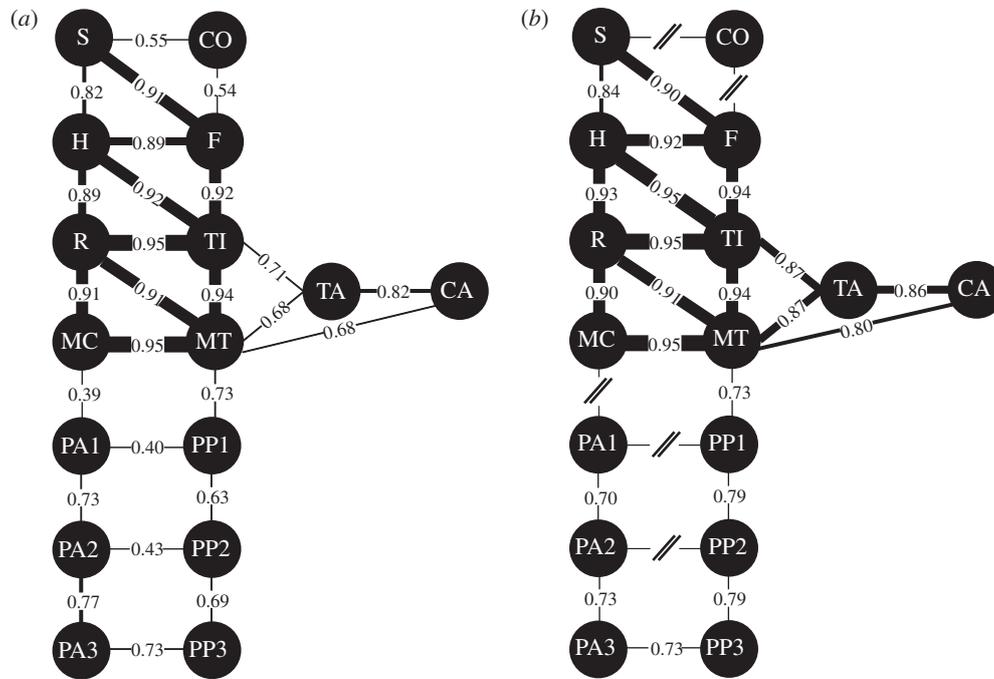


Figure 3. Graphical models of the CR and PLS coefficients obtained on the appendicular bones of horses. The line thickness is proportional to the coefficient values (the boldest lines corresponding to the strongest intensity of covariation). The absence of covariation (non-significant PLS result) is represented by a double slash '//'. S, scapula; H, humerus; R, radio-ulna; MC, metacarpal bone; PA1, proximal anterior phalanx; PA2, middle anterior phalanx; PA3, distal anterior phalanx; CO, coxal bone; F, femur; TI, tibia; TA, talus; CA, calcaneus; MT, metatarsal bone; PP1, proximal posterior phalanx; PP2, middle posterior phalanx; PP3, distal posterior phalanx. (a) CR values, (b) PLS values.

than between girdles/stylopods' (scapula/humerus and coxal bone/femur), between phalanges, and between tarsal bones. The comparison between fore- and hind limbs reveals that there is stronger covariation between scapula and humerus than between the coxal bone and femur. The results also show that the covariation was slightly greater between hind limb long bones (femur/tibia and tibia/metatarsal) than forelimb long bones (humerus/radio-ulna and radio-ulna/metacarpal).

(c) Morphological integration in domestic horses

(i) Serially homologous bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the serially homologous elements in horses (figure 3a). CR and PLS coefficients indicate that there is strong covariation (PLS > 0.90) between serially homologous long bones (humerus/femur, radio-ulna/tibia and metacarpal/metatarsal) with ascending values from the most distal to the more proximal pairs of bones (figure 3). The PLS values indicate that there is no covariation between the serially homologous phalanges (which is concomitant with the low CR values), except the distal ones which are lowly integrated. Finally, there is no significant covariation between scapula and coxal bone, according to the PLS coefficient, which is in agreement with the low CR value.

(ii) Functionally equivalent bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the functionally equivalent elements (figure 3a). All the PLS-associated permutation tests are significant, indicating significant covariation between the functionally equivalent elements (figure 3b). CR and PLS coefficients indicate that there is strong covariation

between the functionally equivalent bones scapula/femur, humerus/tibia and radio-ulna/metatarsal (PLS > 0.90).

(iii) Within-limb connected bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the within-limb connected elements (figure 3a). CR and PLS coefficients indicate that there is strong covariation (PLS > 0.90) between fore- and hind limb long bones (humerus/radio-ulna, radio-ulna/metacarpal, femur/tibia, and tibia/metatarsal) with higher values than between girdles/stylopods, between within-limb phalanges, and between tarsal bones (figure 3). There is no covariation between the coxal bone and femur whereas the scapula and humerus covary to a relatively high degree. Nevertheless, when excluding girdles, the hind limb is more integrated than the forelimb.

(d) Morphological integration in domestic donkeys

(i) Serially homologous bones

All the CR-associated permutation tests are significant (except for the distal phalanges) with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the serially homologous elements (figure 4a). CR and PLS coefficients indicate that there is strong covariation (PLS > 0.90) between radio-ulna/tibia and metacarpal/metatarsal with ascending values from the most distal to the more proximal pairs of bones (figure 4). However, there is no morphological covariation between the humerus and the femur. The PLS values also indicate that there is strong covariation between the serially homologous phalanges (particularly, the proximal and middle ones), which is concomitant with the high CR values. Finally, there is no significant covariation between the scapula and coxal bone,

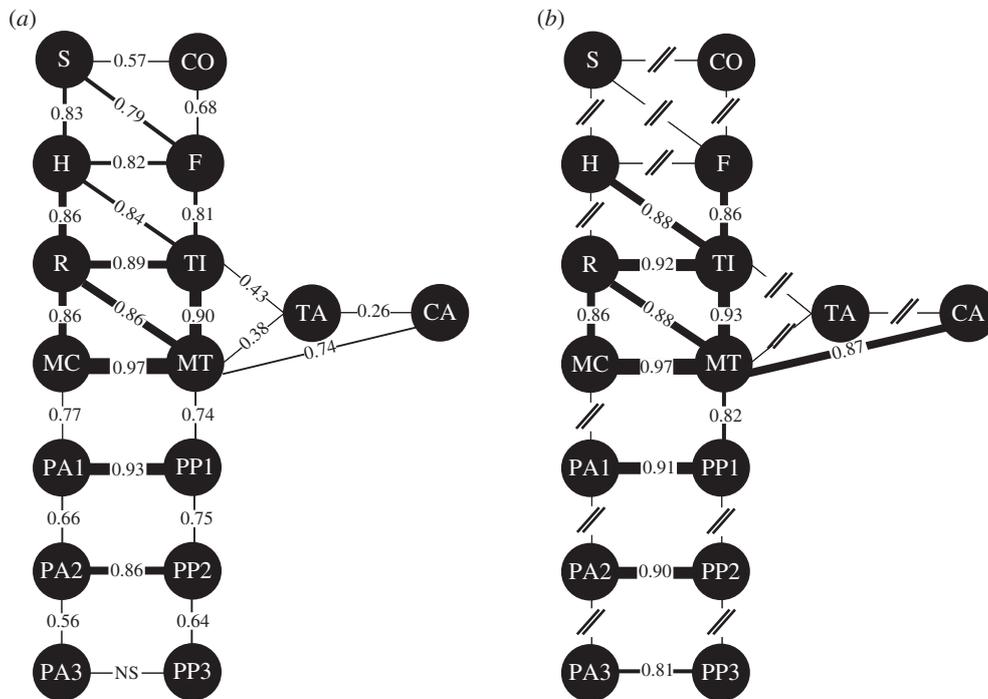


Figure 4. Graphical models of the CR and PLS coefficients obtained on the appendicular bones of donkeys. The line thickness is proportional to the coefficient values (the boldest lines corresponding to the strongest intensity of covariation). The absence of covariation (non-significant PLS result) is represented by a double slash '//'. The absence of modular signal (non-significant CR result) is indicated by 'NS'. S, scapula; H, humerus; R, radio-ulna; MC, metacarpal bone; PA1, proximal anterior phalanx; PA2, middle anterior phalanx; PA3, distal anterior phalanx; CO, coxal bone; F, femur; TI, tibia; TA, talus; CA, calcaneus; MT, metatarsal bone; PP1, proximal posterior phalanx; PP2, middle posterior phalanx; PP3: distal posterior phalanx. (a) CR values, (b) PLS values.

according to the PLS coefficient, which is concomitant with the low CR value.

(ii) Functionally equivalent bones

All the CR-associated permutation tests are significant with CR values lower than one, indicating that the degree of covariation within bones is higher than between bones for all the functionally equivalent elements (figure 4a). CR and PLS coefficients indicate that there is a relatively strong covariation between the functionally equivalent bones humerus/tibia and radio-ulna/metatarsal (PLS > 0.85; figure 4). However, there is no significant covariation between the scapula and femur.

(iii) Within-limb connected bones

All the CR-associated permutation tests are significant with CR values lower than one (figure 4a). This indicates that the degree of covariation within bones is higher than between bones for most of the elements. CR and PLS coefficients indicate that there is no covariation between the forelimb bones, except between the radio-ulna/metacarpal (figure 4). Concerning the hind limb, there is strong covariation (PLS > 0.85) between long bones (femur/tibia and tibia/metatarsal). On the contrary, coxal bone, tarsal bones, and hind limb phalanges are weakly or not integrated with the connected elements.

4. Discussion

(a) Developmental mechanisms of limb morphological covariation

The CR values are lower than one which reveals that within-bone traits covary more with themselves than with other elements. This is concomitant with Hallgrímsson's

developmental hypothesis which suggests that morphological integration is higher within-elements than between-elements [15]. Furthermore, the results demonstrate that covariation between serially homologous long bones is strong in equids, which also corresponds to results for generalized quadrupeds [2,15]. Indeed, it was hypothesized that a significant covariation would exist between serially homologous limb elements due to a shared developmental pathway in tetrapods. This developmental hypothesis implies that any change to an element would impact the serially homologous ones and therefore, in the case of a negative impact, selection would favour the breakdown of the covariation between fore- and hind limbs. For instance, the functional divergence of limbs in humans (for bipedal locomotion) or in apes (for forelimb suspension) has produced reduced integration compared to quadrupedal monkeys [22]. On the contrary, the absence of functional specialization or task division between limbs in equids could explain the strong integration observed between serially homologous elements.

Nevertheless, in spite of the global and strong integration between the serially homologous elements, variation in the intensity of integration was observed along the limbs. First, the results showed that there is no covariation between the girdles. This particularity can be explained by the fact that the coxal bone articulates directly with vertebrae whereas the scapula is suspended by muscles due to the absence of a clavicle. This configuration implies forces being dissipated by the muscles before being transferred to the axial skeleton and, as a result, a more reduced link with the rest of the skeleton. Furthermore, our results also revealed a lower intensity of integration between serially homologous phalanges than long bones. Similar observations were reported by Hallgrímsson *et al.* [2] and Young & Hallgrímsson [15] in their work: murines, minks, and bats all displayed low correlations between the

autopodials whereas they were significant in both quadruped and non-quadruped primates. These previous studies revealed a tendency for covariation patterns to be more variable along the proximo-distal gradient with a breakdown of the covariation between autopodial parts in some taxa. This tendency could be explained by the increase in variability along the proximo-distal gradient of the limb [2]. Indeed, autopods are considered as more variable than the most proximal parts of the limbs not only due to their direct contact with the ground causing a greater sensitivity to environmental pressures, but also due to the proximo-distal direction of limb development [38–41] resulting in the accumulation of variation in the distal parts. Thus, structural and functional mechanisms likely contributed to reshaping the integration patterns between serially homologous girdles and phalanges in equids.

Nevertheless, the strong covariation between the serially homologous long bones confirms that shared developmental factors contribute to structuring equine limb covariation and that integration patterns due to developmental mechanisms are globally preserved as in other quadrupeds [15,18,19,21,22,42].

(b) Functional mechanisms of limb morphological covariation

Besides contributing to reducing morphological covariation between serially homologous bones, functional mechanisms can also reinforce covariation between elements involved in common functional tasks.

(i) Covariation between within-limb connected bones: the signal of shared functional tasks

Our results showed strong morphological integration between anatomically connected elements. Within-limb connected elements share mechanical environments [2] and the morphological covariation between them can be considered as reflecting shared functional requirements [15,22,43,44]. Moreover, the highest levels of integration are located on long bones which are strongly involved in locomotion. This suggests that functional factors are driving the observed integration. In both horses and donkeys, the morphological integration is strong between hind limb long bones (femur, tibia, and metatarsal) as previously observed in generalized quadrupeds [15,22]. As the hind limb is the principal limb generating propulsion in equids [44], the strong morphological covariation between femur, tibia, and metatarsal observed here could signal its functional importance [45–48].

Within-limb covariations also vary along the limbs. Morphological integration between autopodial elements (tarsal bones, metapodials, and phalanges) is globally lower than between more proximal bones. This might at least partly be related to the greater intrinsic variation present in the most distal parts of the limb [2,15]. Furthermore, our results for domestic equids reveal that the integration between girdles and stylopods is low or, in some cases, even absent. The scapula and humerus are globally more integrated than the coxal bone and femur. This difference can be explained by the direct connection of the coxal bone to the trunk which probably engages it in more various functions than the scapula which actively participates in locomotion [15]. However, while the scapula covaries with humerus in horses, it is only with a relatively low intensity and this covariation is absent in donkeys. This can be related to the singular development of the tetrapod scapula which

contains multiple developmental tissues leading to it being described as a relatively independent element [49–51].

(ii) Covariation between functionally equivalent bones: a strongly coordinated locomotion

Functional requirements can also reinforce morphological integration between fore- and hind limb elements. Schmidt & Fischer [21] proposed a model of covariation between functionally equivalent bones (scapula/femur, humerus/tibia, and radio-ulna/tarsal-metatarsal) based on the reorganization of the limbs in therian mammals [23,24]. Our results reveal morphological covariation between function equivalents which supports the hypothesis of Schmidt & Fischer [21] and reveals the role of coordinated locomotion in shaping patterns of covariation. The lower covariation between the scapula and femur was already observed in previous studies [20] and can be explained by differences in developmental processes and the timing thereof [52].

(iii) Covariation between serially homologous bones: an impact of cursorial specialization?

The strongest values of integration were detected between the metacarpal and metatarsal. Moreover, our results show that the intensity of morphological covariation increases from the stylopod to the metapodial bones. This can appear as contrasting when considering the hypotheses proposed by Hallgrímsson *et al.* [2] which state that variation increases from proximal to distal, involving a potential breakdown in integration for the distal-most parts. However Martín-Serra *et al.* [20] obtained results similar to ours for cursorial carnivores with the morphological integration increasing for the distal parts (in the zeugopod relative to the stylopod). Considering that being a 'non-cursor' is an ancestral condition of carnivores, they linked this pattern to a specialization in locomotion which specifically affects the most distal elements. This hypothesis is concomitant with the idea that distal parts are more susceptible to modification by functional factors. Thus, specialization for cursorial locomotion would not only preserve integration patterns partly inherited from developmental mechanisms, but also reinforce them. Similar to cursorial carnivores, equids display an extremely specialized and unidirectional locomotion which is, for many of them, selected for running abilities. Thus, our results are in accordance with the hypothesis of Martín-Serra *et al.* [20] and confirm it for the metapodial elements. This is unsurprising given the strong connection of the metapodial elements to the limb musculoskeletal system through the tendinous insertions of major muscles (i.e. flexor and extensors muscles).

Thus, selection imparted by the functional demands of locomotion reinforces developmental mechanisms and contributes to the increased morphological integration between serially homologous bones, particularly the metapodial bones.

(iv) Impact of functional specialization on integration patterns: comparison between horses and donkeys

In spite of global common tendencies, integration patterns differ between horses and donkeys. We firstly note a lower covariation in donkeys than in horses between fore- and hind limb long bones, particularly between functionally equivalent ones. This could be related to differences in locomotion between the two taxa. Indeed, donkeys are known for being adapted to rough

terrain including mountainous or rocky areas [53]. Locomotion on rough or hilly terrain could imply variable and multidirectional ground reaction forces. By contrast, domestic horses generally display a lower variability of their locomotor repertoire including more linear movements, in general, whether for running or draft. Thus, although a unique function is shared by the fore- and hind limbs in donkeys, the overall greater variability in their locomotion could imply global lower intensity of morphological integration between limbs. The high level of morphological integration between horse limbs is possibly associated with the strongly coordinated locomotion which is, furthermore, under strong artificial selection in horses. The apparent breakdown of covariation between the serially homologous humerus and femur in donkeys could be considered as signalling that the developmental mechanisms for integration do not 'match' the functional requirements [1]. Conversely, the high degree of covariation observed between the serial homologous phalanges in donkeys contrasts with the results obtained for horses. This reveals that selection has not favoured the breakdown of covariation between the homologous phalanges in donkeys, unlike in horses. This result is in agreement with the observed variability in autopod integration between the taxa [15]. Although speculative at this point in time, we here suggest that the strong integration between the distal-most segments in donkeys may be due to strong functional constraints on stability, needed when navigating across rough terrain.

Our results show that there is no covariation between humerus and radius in donkeys whereas these bones are strongly integrated in horses. A lower degree of morphological covariation within the forelimb than the hind limb has already been highlighted for several quadrupeds [15,22]. It has been related to the more reduced functional role in locomotion of the forelimb, considered as optimized for support or braking in most tetrapods [45–48]. The high level of morphological integration between the horse anterior long bones probably reveals that the forelimb plays an important role in horse locomotion.

Globally, our results show that the appendicular skeleton of domestic horses is more integrated than in domestic donkeys. Previous studies have highlighted that the intensity of morphological integration can vary among quadrupeds in relation to the diversity of their activities: thus, limbs of artiodactyls [21] or cursorial carnivores [20] were described as strongly integrated due to the high degree of specialization of their locomotion. Thus, the fact that the horse appendicular skeleton appears as strongly integrated could be related to an intense degree of 'specialization', probably higher than that observed in donkeys. This difference might be firstly explained by natural selection. Indeed, the respective wild ancestors of the domestic horse and donkey originated from different environments, probably involving different locomotor specializations: whereas the African wild ass is described as well-adapted to mountainous terrain [54,55], the Pleistocene horses were particularly abundant in open environments, like steppes, where unidirectional locomotion would be likely under selection [56]. However, we can suppose that the artificial selection to which equids have been subjected to because their domestication has also contributed to shape the integration patterns. Indeed, whereas donkeys were selected primarily for their working capacity, speed (and more broadly unidirectional forces) is the principal subject of artificial selection in horses. Thus, the natural and artificial selection on running ability in

horses could explain the higher morphological integration of their appendicular skeleton, as already suggested for cursorial carnivores [20]. Nevertheless, in the absence of wild specimens, there is no way to disentangle the influence of natural and artificial selection on the morphological integration patterns.

5. Conclusion

Our results on domestic equids are consistent with previously established hypotheses for generalized quadrupeds and reflect the interactions between developmental and functional factors driving morphological integration. In both horses and donkeys, the strong covariation between serially homologous bones signals the preservation of patterns inherited from shared developmental processes. The variation in the intensity of covariation along the limbs can be explained by the influence of structural mechanisms which contributed to reshaping integration patterns. Similarly, functional factors contribute to reshaping patterns and to increasing the covariation between connected bones involved in common functional tasks. Our results also corroborate the model of morphological covariation between functionally equivalent bones. Thus, the strong morphological integration between fore- and hind limbs can be considered as the signal of a highly coordinated locomotion. Moreover, the high degree of covariation between distal parts can be considered as the result of their locomotor specialization.

Our data also demonstrate that the appendicular skeletons of domestic horses and donkeys constitute relatively strongly integrated systems and share a similar structure of covariation. Nevertheless, the limb bones of horses appear as more integrated than those of donkeys which could be related with the high level of specialization of horses. The fact that equids are submitted to a strong artificial selection which has proceeded over short time spans has not reduced the morphological integration, however. Thus, the high degree of morphological integration in the equid skeleton confirms that the constraints imposed by the covariation structure are weak enough to allow morphological changes and evolution [15,57,58]. However, it also raises the issue of the potential impact of artificial selection on integration patterns. Indeed, the high degree of covariation detected in the horse skeleton might suggest that artificial selection could not only preserve integration patterns partly inherited from developmental mechanisms, but also contribute to increasing the morphological integration to match the functional requirements. A study comparing wild and domestic equids would be important in order to assess the impact of artificial breeding. Similarly, future studies exploring differences in covariation patterns between different breeds would also be of interest to better assess the impact of artificial selection on morphological integration in domestic equids.

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