Skull dorsal asymmetries between three different rabbit types signals stress in paedomorpic animals

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Abstract: Fluctuating asymmetry (FA) is frequently considered a consequence of developmental instability (DI), defined as the inability of an organism to adjust its development to an ideal pattern. The underlying hypothesis is that, under ideal conditions, development should produce nearly symmetric final forms. DI is not directly observable and that is why one of the main reasons for studying FA relies on the hypothesis that it may reflect DI. Directional asymmetry (DA), on the other side, occurs when one side is more often larger than the other, that is, the consistent difference between a pair of morphological structures. For zootechnical studies, developmental stress can be an important factor for inferring inadequate management or selective conditions. In this regard, cranial asymmetries of domestic rabbit were compared for examining their FA and DA in order to infere aspects of living and selective conditions. In this study, 69 individuals who belong to different types (46 *toys*, 10 *beliers*, these both types considered as companion, and 13 meat) were investigated by means of geometric morphometric methods. Digital pictures were obtained for each skull on its dorsal aspects and ten two-dimensional landmarks were then putted on both neurocranium and splachnocranium. Results show that here was statistically significant DA in all three types, but only companion rabbits (*toys* and *beliers*) presented significative FA. It is considered that this is a reflect of relatively high developmental stress in the companion animals (*toys* and *beliers*), selected for extreme paedomorphic traits, in comparison to meat type, and also a physiological masticatory lateralization in the species, independently of the aptitude. Probably directionalized asymmetry along the left and right side of the skull in mammals may be more prevalent than once believed.

Key-Words: - Belier, Fluctuating asymmetry, Developmental instability, Directional asymmetry, Paedomorphy, Toy rabbit

1 Introduction

Bilateral asymmetry can be defined as the differences in size or shape between the sides of any organism [1]. Three kinds of asymmetries are normally described [1]: directional asymmetry (DA), which occurs when one side is more often larger than the other, that is, the consistent difference between a pair of morphological structures [2], antisymmetry, which occurs when there is a l ack of a preferential side on which the asymmetry is manifested, and finally, the fluctuating asymmetry (FA). FA is manifested randomly in a population, without side preference [1].

FA is frequently considered a consequence of developmental instability (DI), defined as the inability of an organism to adjust its development to an ideal pattern [3]. The underlying hypothesis is that, under ideal conditions, development should produce nearly symmetric final forms [4]. DI is not directly observable and that is why one of the main reasons for studying FA relies on the hypothesis that it ma y reflect DI [4]. So exposure to inadequate environmental conditions results in DI, which can be measured through FA [1]. The hypothesis relies on assuming that an organism that fares well is less susceptible to small random developmental errors [4].

FA in cranial morphology arises when a given nonmetric trait shows different grade expression between antimeres, or is expressed in one side and not on the other [5]. This work aims to test FA differences, as a measure of DI, between three different types of rabbits, e.g. *toy*, *belier* and meat. Two former types are bred as pets, and they present clear paedomorphic appearance, that is, retention of early developmental forms into adulthood [6]. There are very few researches of paedomorphosis in domestic animals [7]. Here we report one such case, where extreme paedomorphy involves domestic rabbit, arising some hypothesis that can causes DI.

2 Materials and methods

2.1 Sample

A sample of 69 rabbit corpses were collected in the farm, belonging to *toy* type (n=46), *belier* type (n=10) and meat type (n=13). At the laboratory room, corpses were sexed and measured (body length) and the heads were excised. The defleshing process was done naturally using scavenging beetles and flies. Once this process was completed, skulls were macerated with water, disarticulated and finally whitened with hydrogen peroxide and finally dried at room temperature.

2.2 Photographs and landmark data

Digital photographs of each skull on its dorsal face were obtained, the focal axis of the camera being parallel to the aspect. The camera was attached to a column with an adjustable arm, and above a grid baseboard for measure reference. Imaging capture was performed with a Nikon® D70 digital camera (Nikon Inc., Tokyo, Japan) (image resolution 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28 to 200-mm telephoto lens (Nikon Inc., Tokyo, Japan). Samples are currently deposited in the University of Lleida collection, gathered by first author. The skull morphology was described by a set of 5 pairs of landmarks and semilandmarks (Fig. 1) assumed to be homologous and topologically equivalent. Landmarks used in this study were primarily chosen to describe major skull regions (both neurocranium as splanchnocranium) as well as parts of particular morphofunctional interest and we consider they give a good representation of the overall skull shape and in a way that allow us to see important features of asymmetry.

Each landmark was digitalized two times independently in each skull by the third author to allow for estimating replicability. The captured images were transformed to tpsUtil software v. 1.40 [8]. The Cartesian *X Y* coordinates of all landmarks were digitized using TpsDig, v. 2.26 software [8]. This set was further standardized by the Generalized Procrustes Analysis (GPA). GPA begins by reflecting landmark configurations from one of the sides and superimposing them by their centroid (midpoint of a configuration of anatomical landmarks). Then, each landmark configuration was rotated such that the squared distances between homologous landmarks were minimized and Procrustes coordinates obtained [9]. As a result of all of these calculations, the distances between the superimposed configurations of left and right were obtained. Replicability of Procrustes coordinates was then analysed by a two-way NPMANOVA (Non-Parametric-Multivariate-Analysis-Of-Variance) with individuals and replicas as factors. To detect asymmetries, the interaction between the side of the body and the individual identity corresponding to FA, the effect of the side corresponding to DA. A multivariate regression using body length as independent variable and FA values as dependent ones, was performed. Finally, a Principal Component Analysis (PCA). Analysis of data was done with software MorphoJ [10] and PAST [11].

2.3 Ethics Statement

This study was carried out in corpses from naturally dead animals and in any case they were euthanized, so no Ethics Committee agreement was considered to be unnecessary.

3 Results

The individual amount of variation exceeded the replicas variation (table 1) suggesting that measurement error (that component of the overall variance due to imprecision of measurements in this study) was random and did not affect the outcome of asymmetry analyses. The values in the table 2 reveal high inter-individual variation in skull shapes among the specimens in all types, being FA the main source of asymmetric variation but with significative levels only for *toys* (p=0.022) and *beliers* (p<0.0001). FA was positively regressed with body length (R^2=0.133, Wilk's λ=0.0931, $F_{20,91}$=44.29, p<<0.001). DA appeared statistically significative for all types (p<0.05). First principal component of PCA for FA companion rabbits, which explained a 56.18% of the total observed variance, showed a clear tendency towards a lengthening of face, with no increase of width, and a narrowing of skull, with no increase of length (Figure 2).

4 Discussion

Fluctuating asymmetry (FA), small, random deviation in the development of both sides of a bilateral symmetric structure, has been extensively used as an indicator of stress and/or fitness [12]. So it has been hypothesized that FA should correlate positively with stress (i.e. disruptions of normal development due to genetic and/or environmental cues are expected to increase the asymmetry level) and negatively with fitness (i.e. well-faring organisms are expected to display both higher fitness and lower developmental instability (DI). DI is a form of phenotypic plasticity, which is contrary to stabilizing selection and therefore reduces population fitness [12]. DI can signal the organism about unfavourable environmental conditions. Wide and short jaws, large head, and a flat face -typical traits of companion rabbits (*toy* and *belier* rabbits, selected for these extreme values)- can be considered as a "handicap" for a normal development (limitations to jaw aperture, respiratory obstructions….), so FA appears significative for this type of rabbits.

DA reflects a consistent bias of a character within a population toward asymmetric development, implying the existence of an ideal left side that is different from the ideal right side for all individuals in a population. DA is not expected to reflect DI, as it has an unknown genetic component. In our study, detected DA is supposed to be due to lateralization of mastication, as some masticatory muscles attach on the dorsal aspect of the skull (e.g. superficial temporalis and posterior deep masseter) [13]. This lateralization has been described in other domestic mammals, such horses [14] and wild boars [15]. Many instances of more subtle quantitative instances of DA have also been observed, such as testicle position and size in mammals and wing size and shape in Diptera [2]. Although both systemic factors (e.g. hormonal regulation, nutrient availability, genetic variation) and local (e.g. mechanical stress) could contribute to AD, the available evidence suggests the role of biomechanical forces as the main cause of differences in size and shape in the case studied. In this sense, the higher degree of asymmetry of one cranial shape in respect to the other could be attributed to the effect of bone remodelling stimulated by the biomechanical forces, and would reflect different functional demands due to chewing.

5 Conclusions

Cranial morphology fluctuating asymmetry was analyzed as an indicator of developmental instability in 3 types of domestic rabbits. Masticatory lateralization can be considered as physiological (e.g. normal) for the species and so not linked to a determined type or aptitude. The results presented here may improve future exploration of the potential link between FA and fitness and provide a better indicator of performance in domestic rabbits. This preliminary study indicates the companion sample (*toy* and *belier* rabbits) may be subjected to higher levels of FA than meat ones. If large values of FA should predict lower fitness, bigger animals would present less fitness. To our knowledge this is the first documented research of paedomorphosis playing a role in rabbit.

6. Authors' contributions

PMPC designed the experiments, analysed the data and wrote the paper. AM and KS performed completely the field study.

7. Competing interests

Authors have no competing interests.

References:

[1] L. M. Marado and A. M. Silva, 'Dental and oral nonmetric traits in a Coimbra reference sample: testing intrasample chronological and spatial variation', *Archaeol. Anthropol. Sci.*, vol. 11, no. 2, pp. 1–13, 2016.

[2] A. J. R. Carter, E. Osborne, and D. Houle, 'Heritability of Directional Asymmetry in Drosophila melanogaster', *Int. J. Evol. Biol.*, pp. 1–7, 2009.

[3] J. C. Auffray, V. Debat, and P. Alibert, 'Shape asymmetry and developmental stability', in *On growth and form: spatio-temporal pattern formation in biology*, no. 1, J. C. M. Mark A.J. Chaplain, G.D. Singh, Ed. New York: John Wiley and Sons Ltd, 1999, pp. 309–324.

[4] J. A. Cocilovo, H. H. Varela, and S. Quevedo, 'La asimetría bilateral y la inestabilidad del desarrollo', *Rev. argentina Antropol. Biol.*, vol. 8, no. 1, pp. 121–144, 2006.

[5] L. Van Valen, 'A study of fluctuating asymmetry', *Evolution (N. Y).*, vol. 16, pp. 125–142, 1962.

[6] W. S. Armbruster, J. Lee, M. E. Edwards, and B. G. Baldwin, 'Floral paedomorphy leads to secondary specialization in pollination of Madagascar Dalechampia (Euphorbiaceae)', *Evolution (N. Y).*, vol. 67, no. 4, pp. 1196–1203, 2013.

[7] F. A. M. Tuyttens *et al.*, 'Measuring fluctuating asymmetry in fattening rabbits: a valid indicator of performance and housing quality?', *J. Anim. Sci.*, vol. 83, no. 11, pp. 2645–2652, 2005.

[8] F. J. Rohlf, 'The tps series of software', *Hystrix, Ital. J. Mammal.*, vol. 26, no. 1, pp. 9–12, 2015.

[9] C. P. Klingenberg, 'Analyzing Fluctuating Asymmetry with Geometric Morphometrics: Concepts, Methods, and Applications', *Symmetry (Basel).*, vol. 7, pp. 843–934, 2015.

[10] C. P. Klingenberg, 'MorphoJ: An integrated software package for geometric morphometrics', *Mol. Ecol. Resour.*, vol. 11, no. 2, pp. 353–357, 2011.

[11] Ø. Hammer, D. A. T. Harper, and P. D. Ryan, 'PAST v. 2.17c', *Palaeontol. Electron.*, vol. 4, no. 1, pp. 1–229, 2001.

[12] G. de Coster *et al.*, 'Fluctuating Asymmetry and Environmental Stress: Understanding the Role of Trait History', *PLoS One*, vol. 8, no. 3, pp. 1–9, 2013.

[13] P. J. Watson *et al.*, 'Masticatory biomechanics in the rabbit: a multi-body dynamics analysis', *J. R. Soc. Interface*, vol. 11, no. 99, pp. 1–14, 2014.

[14] P. M. Parés-Casanova, 'Size asymmetries in equine upper molar series', *ECORFAN J.*, vol. 5, no. 13, pp. 2055–2069, 2014.

[15] P. M. Parés-Casanova, 'Existence of mandibular directional asymmetry in the European wild boar (Sus scrofa Linnaeus, 1758)', *J. Morphol. Sci.*, vol. 31, no. 4, pp. 1–5, 2014.

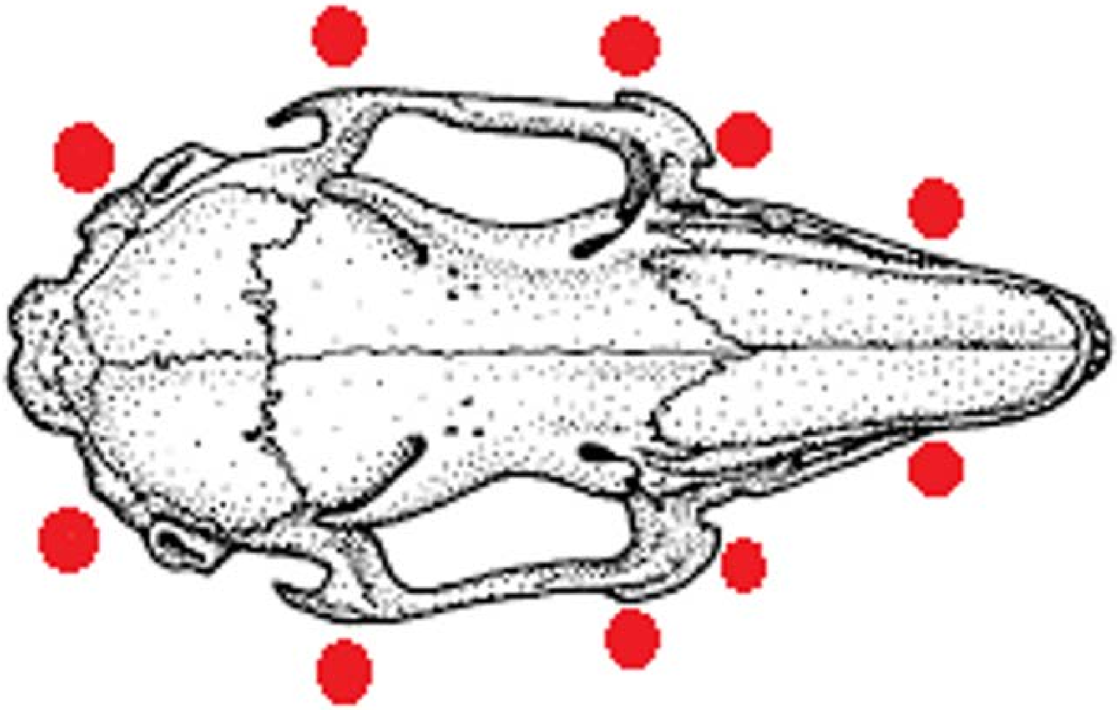

Figure 1. Set of landmarks and semilandmarks (5 pairs) used for this study. They covered both the neurocranium and the splanchnocranium, and were assumed to be homologous and topologically equivalent.

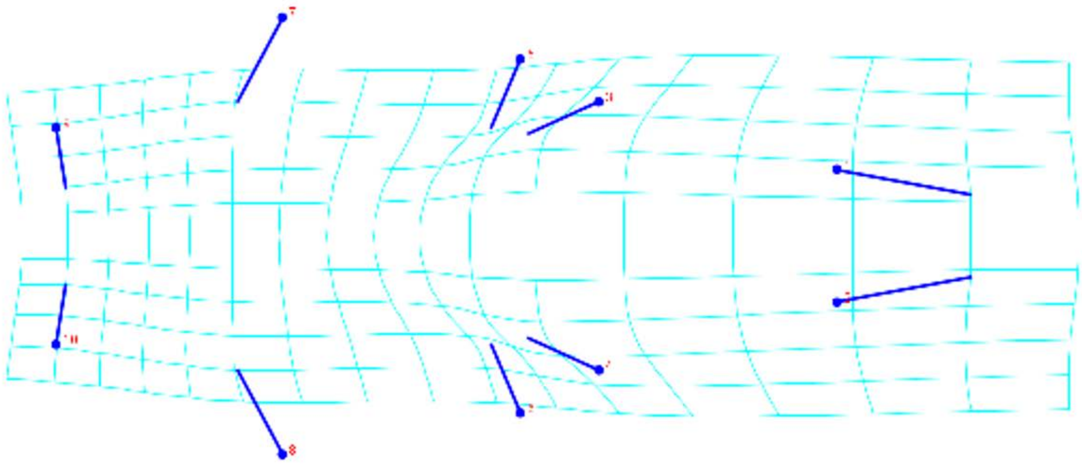

Figure 2. Deformation grid for first principal component of Principal Component Analysis for Fluctuating Asymmetry in companion rabbits, which explained a 56.18% of the total observed variance. It showed a clear tendency towards a lengthening of face (right), with no increase of width, and a narrowing of skull (left), with no increase of length.

Table 1. Two-way NPMANOVA (Non-Parametric-Multivariate-Analysis-Of-Variance), using 9,999 permutations and Euclidean distances for skull Procrustes coordinates (dorsal aspect), with individuals and replicas as factors for 69 rabbits. The individual amount of variation exceeded the replicas variation suggesting that measurement error was random and did not affect the outcome of asymmetry analyses.

Source	Sum of squares	Degrees of Freedom	Mean square	*F*	*p*
Individual	2.37E+29	63	3.76E+27	12.169	0.0328
Replica	1.57E+27	1	1.57E+27	0.5085	0.7872
Interaction	1.43E+29	63	2.27E+27	0.7350	0.9917
Residual	3.96E+29	128	3.09E+27		
Total	7.77E+29	255			

Table 2. Two-way NPMANOVA (Non-Parametric-Multivariate-Analysis-Of-Variance), using 9,999 permutations and Euclidean distances for skull Procrustes coordinates (dorsal aspect), with individuals and replicas as factors for each rabbit type ("toy" type (n=46), "belier" type (n=10) and meat type (n=13)). The interaction between the side of the body and the individual identity corresponded to FA, the effect of the side corresponded to DA.

Source	Sum of squares	Degrees of Freedom	Mean square	*F*	*P*
Individual (toys)	0.10340918	0.0002872477	360	9.78	<0.0001
Side	0.00298214	0.0003727679	8	12.69	<0.0001
Interaction	0.01057167	0.0000293658	360	1.20	0.0226
Residual	0.01806002	0.0000245381	736		
Individual (beliers)	0.01277904	0.0001774867	72	4.68	<0.0001
Side	0.00098691	0.0001233642	8	3.26	0.0032
Interaction	0.00272856	0.0000378967	72	2.65	<0.0001
Residual	0.00228967	0.0000143105	160		
Individual (meat)	0.01117517	0.000116408	96	3.91	<0.0001
Side	0.00147104	0.000183879	8	6.17	<0.0001
Interaction	0.00286076	0.000029799	96	1.30	0.0633
Residual	0.00478202	0.000022990	208		