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Fluctuating asymmetry in metric traits; a practical example of calculating asymmetry, measurement error, and repeatability

Thea Ø. Bechshøft[1,2], Frank F. Rigét[2], Øystein Wiig[1] & Christian Sonne[2,*]

[1)] *Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, N-0562 Oslo, Norway*
[2)] *Department of Arctic Environment, National Environmental Research Institute, University of Aarhus, P.O. Box 358, DK-4000 Roskilde, Denmark (*corresponding author's e-mail: csh@dmu.dk)*

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We present an example of background statistics in studies of fluctuating asymmetry (FA); calculations of asymmetry, measurement error (ME), and repeatability. Nine bilateral metric traits in skulls and lower jaws of 691 East Greenland and Svalbard polar bears (*Ursus maritimus*) were measured twice, and examined for asymmetry. The skulls were collected in the period 1892–2004. In this study, 2.0% of the FA data were identified and treated as outliers, which is less than in comparable studies. FA for each trait amounted to 0.1%–3% of the average size of the corresponding trait. The magnitude of FA generally increased with trait size. For every trait measured, ME was found to be smaller than FA. The repeatability of the traits was inversely proportional to ME. Five of the nine traits had a repeatability of 90% or more, which is similar to what has been reported in other studies.

Introduction

Fluctuating asymmetry (FA) refers to small, random deviations from the ideal morphological symmetry and is measured as the absolute difference between a trait on the left- and right-hand side of a bilaterally symmetrical organism (Møller & Swaddle 1997, Palmer & Strobeck 2003a). FA is often taken as a measure of developmental stability (DS), "the ability to attain equal development under the given circumstances" (Zakharov 1992), and its counterpart developmental instability (DI). In principle, DS reflects the organism's ability to buffer its development against disturbance (Møller & Swaddle 1997). The more fit the genotype of an organism, and the less stress from the surrounding environment it is exposed to, the higher DS and thus lower FA it is expected to have (Stige 2004). Higher levels of FA have been connected with increased population density (Zakharov *et al.* 1997), social stress (Valetsky *et al.* 1997, Gibbs & Breuker 2006), sexual selection (Mazzi *et al.* 2003, Voigt *et al.* 2005), nutritional stress (Pravosudov & Kitaysky 2006), heat stress (Siegel *et al.* 1977, Petavy *et al.* 2006), disease and parasitic stress (Møller 2006), and genetic factors such as increased levels of hybridization and

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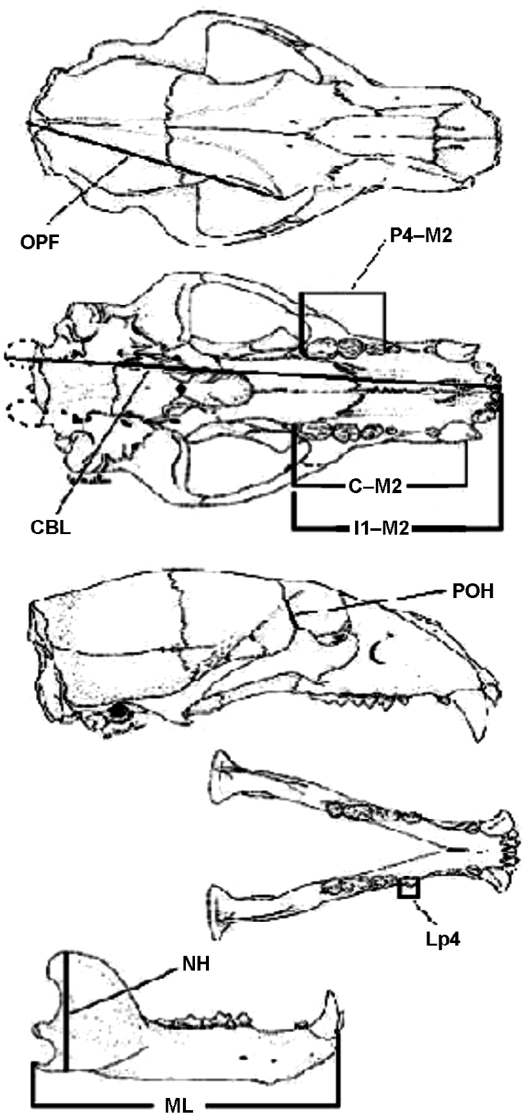

Fig. 1. Nine metric traits (*see* Table 1) measured on skulls of East Greenland and Svalbard polar bears (*Ursus maritimus*). Figure modified from Amstrup and DeMaster (1988).

heterozygosity (Borrell *et al*. 2004, Andersen *et al*. 2006). FA studies have been conducted on metric and meristic traits of a multitude of organisms (Zakharov & Yablokov 1990, Blagojevic & Vujosevic 2004, Vilisics *et al*. 2005, Andersen *et al*. 2006, Green & Lochmann 2006, Pelabon *et al*. 2006, Bechshøft *et al*. 2008).

A robust methodology is essential in FA studies to avoid serious flaws in the analyses (Merilä & Björklund 1995, Stige 2004). One of the greatest challenges in working with FA is getting the measurement error (ME) as small as possible (Merilä & Björklund 1995, Palmer & Strobeck 2003a, Stige 2004). Replicate and independent measurements are important, so as to be able to distinguish between FA and ME in the analyses (Palmer 1994, Merilä & Björklund 1995, Palmer & Strobeck 2003a). Replicate measurements of the chosen traits provide the basis for running a two-way mixed model ANOVA, from which descriptors of FA, ME, and repeatability can be calculated (Palmer & Strobeck 2003a). The aim of this paper is to provide an example of some of these, often unpublished, background statistics in asymmetry studies.

Materials and methods

Sample

A sample of 300 polar bear skulls from East Greenland (held at the Zoological Museum, University of Copenhagen), and 391 polar bear skulls from Svalbard (held at the Natural History Museum, University of Oslo) were examined. The skulls were collected during the period 1892–2004 (East Greenland, approx. 61°–82°N, 10°–42°W) and 1950–2004 (Svalbard, approx. 74°–81°N, 10°–35°E). Many of the skulls were more or less damaged, and thus the entire range of measurements could not be taken on all skulls.

Measurements

The metric traits were measured on the right (*R*) and left (*L*) side of each skull, using digital callipers (Mitutoyo, Mitutoyo Corporation, Japan), to the nearest 0.04 mm. The traits were measured twice on each skull, always by the same person, and never twice on the same day. Nine metric bilateral traits were measured in order to estimate the level of FA in the skulls (Table 1 and Fig. 1).

Statistical analyses

All statistical analyses were performed using

SPSS (ver. 13.0). The significance level was set to $p = 0.05$ unless other is specified.

Outliers

Possible outliers were identified visually from scatter plots as suggested by Palmer and Strobeck (2003b). The outliers were then tested and removed according to Grubbs' test (Grubbs 1969, Palmer & Strobeck 2003b).

FA, ME, and repeatability

A two-way mixed model ANOVA (with $|R - L|$ as dependent variable, individual as random explanatory variable, side as fixed explanatory variable, and the interaction between individual and side) was applied to the replicate measurements in order to estimate the magnitude of FA relative to ME. The MS_{SI} (mean squares of the sides × individuals interaction) and MS_{error} (mean squares of the variance of the repeated measurements [error]) from the two-way mixed model ANOVA were furthermore used to estimate FA excluding ME in mm (FA10a), MS_{error} as a percentage of MS_{SI} (ME3), repeatability (ME5), average difference between the repeated measurements (in mm)(ME1), FA including ME (in mm) (FA4a), ME1 as a percentage of FA4a, and FA1 mean ± SE (the FA index ($|R - L|$) used throughout the study (in mm) (*see* Palmer 1994, Palmer & Strobeck 2003a, 2003b)).

Results

Outliers

A total of 126 measurements (R and L) were excluded from the statistical analysis. This corresponds to 2.0% of all measurements taken.

FA, ME, and repeatability

ME was found to be smaller than FA in all nine traits (Table 2). FA10a gives the measured FA ($|R - L|$), excluding ME, in mm, and ranged between 0.14 and 4.46 mm. The error variance contributed from 0.34% to 13.12% of the total variance between sides (ME3), except for Lp4 with 28.77%. The generally low values of ME3 were reflected in the high repeatability (ME5) of the traits, which ranged between 77% and 99%, except for trait Lp4 with 55%. Expressed in another way, the average difference between the repeated measurements (ME1) made up between 5.85% and 53.64% of FA4a (FA including ME, in mm). FA10a (FA excluding ME) was always lower than FA4a (FA including ME). The differ-

Table 1. Definition of the nine metric traits measured on skulls of East Greenland and Svalbard polar bears (*Ursus maritimus*).

Trait	Definition
Skull	
OPF	Maximal distance between the opistokranion and the postorbital process of the frontal bone.
CBL	Condylobasal length. The maximal distance between the anterior margin of the alveole of the 1st incisor and the anterior margin of the occipital condyles.
P4–M2	Length from the anterior margin of the 4th premolar to the posterior margin of the 2nd molar.
C–M2	Length from the anterior of the alveole of the canine, to the posterior of the alveole of the 2nd molar.
I1–M2	Length from the anterior of the alveole of the 1st incisor, to the posterior of the alveole of the 2nd molar.
POH	Postorbital height. The minimal distance between the postorbital process of the frontal bone and the frontal process of the zygomatic arch.
Lower jaw	
Lp4	Length of the 4th premolar of the lower jaw.
ML	Mandible length. The maximal distance between the anterior margin of the mandibular symphysis and the posterior margin of the angular process.
MH	Mandible height. The maximal distance between the margin of the angular process and the coronoid process.

Table 2. Results from the two-way mixed model ANOVA (sides as fixed factor and individuals as random factor) on nine metric traits measured twice on skulls of East Greenland (n = 300) and Svalbard (n = 391) polar bears (*Ursus maritimus*), and descriptors of FA (fluctuating asymmetry) and ME (measurement error) according to Palmer and Strobeck (2003a): MS_{SI} (mean squares of the sides × individuals interaction), MS_{error} (mean squares of the variance of the repeat measurements [error]), FA10a (FA excluding ME, in mm), ME3 (MS_{error} as a percentage of MS_{SI}), ME5 (repeatability), ME1 (average difference between the repeated measurements, in mm), FA4a (FA incl. ME, in mm), ME1 as a percentage of FA4a, and FA1 mean ± SE (the FA index ($|R - L|$) used throughout the study, in mm).

Descriptor	Trait								
	OPF	CBL	P4–M2	C–M2	I1–M2	POH	Lp4	ML	MH
MS_{SI} [a]	22.08	31.32	1.10	1.78	1.71	0.98	0.11	2.14	3.72
MS_{error} [b]	1.08	0.11	0.02	0.23	0.14	0.02	0.03	0.16	0.20
FA10a [c]	3.66	4.46	0.83	0.99	0.14	0.78	0.22	1.12	1.50
ME3 [d]	4.89	0.34	1.91	13.12	8.17	2.04	28.77	7.66	5.44
ME5 [e]	0.91	0.99	0.96	0.77	0.85	0.96	0.55	0.86	0.90
ME1 [f]	0.83	0.26	0.12	0.39	0.30	0.11	0.14	0.32	0.36
FA4a [g]	3.75	4.47	0.84	1.06	1.04	0.79	0.26	1.17	1.54
ME1 as a percentage of FA4a	22.11	5.85	13.81	36.21	28.58	14.29	53.64	27.67	23.32
FA1 mean ± SE [h]	1.56 ± 0.06	1.04 ± 0.04	0.74 ± 0.03	1.04 ± 0.03	0.95 ± 0.03	0.78 ± 0.03	0.26 ± 0.01	1.40 ± 0.05	0.95 ± 0.03

a) Mean squares of the sides × individuals interaction of the two-way mixed model ANOVA.

b) Mean squares of the variance of the repeat measurements (error), obtained from the two-way mixed model ANOVA.

c) $FA10a = 0.798\sqrt{(MS_{SI} - MS_{error})}$. In mm.

d) ME3: MS_{error} as a percentage of MS_{SI}.

e) $ME5 = (MS_{SI} - MS_{error})/[MS_{SI} + \mathbf{(2 - 1)}MS_{error}]$.

f) $ME1 = 0.798\sqrt{MS_{error}}$.

g) $FA4a = 0.798\sqrt{MS_{SI}}$.

h) FA1: $|R - L|$ of the replicate measurements.

ence between these two indices represents the contribution ME made to the FA. FA differed between traits, but also varied depending on how it was computed. Estimates derived from variances (FA10a and FA4a) generally suggested greater FA variation among traits than the estimate derived from the mean absolute deviation (FA1) (Palmer & Strobeck 2003b).

Discussion

Outliers

In this study, 2.0% of the FA data were identified and treated as outliers. In comparison, Ahtiainen *et al*. (2003) identified 2.1% of their FA data as outliers, and of the data from Crespi and Vanderkist (1997), Palmer and Strobeck (2003b) identified 5.7% as outliers.

FA, ME, and repeatability

For every trait measured, ME was found to be smaller than the FA. The magnitude of FA generally increased with trait size, as expected (Palmer & Strobeck 1986, Leung 1998).

FA for each trait amounted to roughly 0.1%–3.0% of the average size of the corresponding trait (Table 2), which is on average slightly higher than the 1% predicted by Palmer (1996). ME1 gives the measurement error for each trait, but is quite dependent on trait size. When comparing ME between traits, the dimensionless estimator ME1 as a percentage of FA4a is probably better suited. The premolar of the lower jaw (Lp4) appears to be a poor trait for measuring FA, with ME making up 53.6% of the FA (FA4a). On the other hand, the ME of the second tooth trait (P4–M2) only made up 13.8% of the FA (FA4a), despite there being larger room for error here, as the individual teeth were not always very solidly sat in their position in the jaw and hence could be rocked in all directions. Perhaps the one premolar (Lp4) is subjected to a different kind of wear than the row (P4–M2) (Sonne *et al*. 2007), which makes its edges harder to define. The comparatively high error rates of traits C–M2 (36.2%) and I1–M2 (28.6%) could be attributed to difficulty in determining the border of the alveoles, especially the alveole of the canine tooth. The values of ME1 as a percentage of FA4a were all within the same range as found for the traits in Crespi and Vanderkist (1997) as calculated by Palmer and Strobeck (2003b), and those reported by Palmer and Strobeck (1986).

The repeatability (ME5) of the traits was inversely proportional to ME1 as a percentage of FA4a. The harder the traits were to replicate in measurements, the higher the ME. Five of the nine traits had a repeatability of 90% or more (traits OPF, CBL, P4–M2, POH, and MH). Trait Lp4 had the lowest repeatability (55%), which supports the notion of this as a poor trait for FA measurements. The repeatability of the remaining traits (ML, I1–M2, and C–M2) ranged between 77% and 86%. When compared with the results of repeatability in the traits measured by Crespi and Vanderkist (1997) (four traits, range 38%–99.5%) as calculated by Palmer and Strobeck (2003b), and the results of repeatability reported in Kark (2001) (two traits, range 91%–99%), Pertoldi *et al*. (1997) (four traits, range 96.8%–98.2%) and Pertoldi *et al*. (2000) (two traits, range 89%–93%), the results of the present study seem acceptable.

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