Fluctuating asymmetry of red deer antlers negatively relates to individual condition and proximity to prime age

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Abstract

After about 50 years of research on fluctuating asymmetry (FA) as a reliable indicator of both individual quality and environmental stress, the enthusiasm is beginning to decline. The findings of many studies are inconsistent and the relationship between FA and stress appears both weaker and more complex than first thought. To provide clarification of the debate, new studies should use more efficient and unified statistical protocols, large sample sizes and joint analysis of several related traits. In addition, fitness–FA associations should be tested at the individual level, in different populations and under different environmental conditions. To achieve these criteria, we describe a 9-year study in which we measured six antler traits of 3 000 Iberian red deer, *Cervus elaphus hispanicus*, from three study areas in southwest Spain. Males were harvested during hunting activities and measured, weighed and aged postmortem. We found evidence of correlations between traits in FA, an association between asymmetry and stress conditions and a weak but significant negative relationship between FA and fitness surrogates (body mass and antler size), thus supporting some assumptions of the FA hypothesis. As also predicted by theory, antler traits of less functional importance were more asymmetric and more sensitive to stress than those directly used in fighting behaviour. The relationship between age and antler asymmetry was U-shaped, suggesting an effect of sexual selection on antler development in favour of larger and more symmetrical antlers during prime age.

Keywords: antlers; fluctuating asymmetry; Iberian red deer; intrasexual weapons; ex traits; sexual selection

Developmental stability is the ability of an individual genome to buffer its developmental process against intrinsic, ‘random noise’. Many genetic or environmental factors are supposed to disrupt developmental stability and/or increase noise, producing, as a consequence, lower development precision in morphological traits.

Fluctuating asymmetry (FA; small, random deviations from a prior expectation of symmetric development in bilateral traits: Ludwig, 1932, Van Valen, 1962) has been hypothesized to be the phenotypic outcome of developmental instability (DI) and, therefore, a good candidate for assessing DI at different magnitudes of environmental stress (reviews in Parsons, 1992, Clarke, 1995, Møller and Swaddle, 1997, Ditchkoff et al., 2001, Palmer and Strobeck, 2003, Swaddle, 2003, Van Dongen, 2006). Furthermore, because FA can reliably reflect the stress experienced during development, it has been suggested that it could be related to (or predict) individual fitness (Møller 1990; reviewed in Swaddle 2003). If so, symmetry of bilateral sexual traits (both weapons and ornaments) may play an important role in sexual selection because symmetric individuals might have an advantage over asymmetric competitors during mate choice or intrasexual competition (Møller, 1990, Møller, 1992, Møller, 1993;

reviewed in Møller and Swaddle, 1997, Møller and Thornhill, 1998, Thornhill and Møller, 1998, Swaddle, 2003).

However, despite numerous studies on fluctuating asymmetry over a period of 30 years, elucidation of the meaning and consequences of the process is still very poor. Additionally, interest in evolutionary studies of FA has decreased in the past 5 years (see review in Van Dongen 2006). A possible explanation for the decline in FA research is the emergence over time of a cumbersome and complex methodology. Furthermore, studies on FA need a large sample size (Palmer, 1999, Bjorksten et al., 2000, Kruuk et al., 2003, Babbitt et al., 2006) and a rigorous control of measurement errors (Björklund and Merilä, 1997, David et al., 1999, Van Dongen et al., 1999, Bjorksten et al., 2000, Palmer and Strobeck, 2003). Another reason for the decline is the enormous heterogeneity of the results found to date, indicating that the relationship between FA and stress (environmental disturbance) should be both weaker and more complex than first thought (Van Dongen 2006).

Some studies have established an association between fluctuating asymmetry and fitness indicators (reviewed in Møller, 1997, Møller and Swaddle, 1997, Ditchkoff et al., 2001, Lens et al., 2002, Palmer and Strobeck, 2003, Swaddle, 2003). Other works have reported correlations between mate choice or male–male competition and asymmetry (meta-analyses: Møller and Thornhill, 1998, Thornhill and Møller, 1998). However, there have also been numerous negative results, and a series of reviews questioning previous studies and meta-analyses (e.g. Rowe et al., 1997, Clarke, 1998a, Houle, 1998, Palmer, 1999, Simmons et al., 1999, Bjorksten et al., 2000, Borges, 2000, Carchini et al., 2000, Carchini et al., 2001, Kruuk et al., 2003).

The aim of this study was to analyse a new data set on asymmetry of a conspicuous and complex sexual trait: the antlers of Iberian red deer, *Cervus elaphus hispanicus*. Studies on ungulate antler/horn asymmetry are good examples of contrasting results (in support of the FA hypothesis: Solberg & Sæther 1993 for moose, *Alces alces*; Malyon and Healy, 1994, Putman et al., 2000 for fallow deer, *Dama dama*; Møller et al. 1996 for gemsbok, *Oryx g. gazella*; Markusson & Folstad 1997 for reindeer, *Rangifer tarandus*; Pélabon & Van Breukelen 1998 for roe deer, *Capreolus capreolus*; Stenström 1998 for red deer, *Cervus elaphus*; Ditchkoff et al. 2001 for white-tailed deer, *Odocoileus virginianus*; not supporting the hypothesis: Hayden et al. 1994 for sika deer, *Cervus nippon*; Stenström, 1998, Pélabon and Joly, 2000 for fallow deer; Stenström 1998 for moose; Kruuk et al., 2003, Bartoš and Bahbouh, 2006, Bartoš et al., 2007 for red deer).

Nevertheless, antlers of red deer constitute an appropriate subject for FA studies. They are renewable and exaggerated sexual traits and thus good candidates for indicating either male quality or stress experienced during the annual antler development (reviewed in Geist 1998). In addition, antlers are primarily weapons and their size in red deer is an age-related variable that is positively correlated with mating success, although there is no evidence for female choice based on antler features (Clutton-Brock et al., 1982, Kruuk et al., 2002, Malo et al., 2005, Bartoš and Bahbouh, 2006).

Our main contribution relies on having the necessary data for statistical analyses to be sufficiently robust: a large sample size of males from three study areas and measured in different years and conditions. Males were not only weighed and measured but aged also, which is essential for FA studies in ungulates (Kruuk et al. 2003). As some authors have recently pointed out (Clarke, 2003, Van Dongen, 2006), to advance in the debate, fitness and FA associations should be tested at the individual level, in different populations and under different environmental conditions. In addition, we have measured different antler traits, some of which (see Methods) can be considered independent traits of a complex character. This allows for a more robust estimate of the underlying developmental instability of an individual (Leung et al., 2000, Palmer and Strobeck, 2003, Van Dongen, 2006). Furthermore, data from different traits also permit us to test the suggestion that some traits may be more vulnerable to the effects of stress than others (reviewed in Bjorksten et al., 2000, Swaddle, 2003, Van Dongen, 2006).

In this study we tested the following specific hypotheses of the fluctuating asymmetry paradigm on red deer antlers: (1) there is a positive correlation between different traits in the absolute value of FA at the individual level, (2) variation of antler FA among individuals relates to indicators of fitness such as body mass or antler size, (3) variations in antler FA among groups of males are associated with differences in age or environmental conditions (see Methods), (4) some antler traits are more affected than others by age or environmental factors and (5) the FA level is also trait specific because some parts of the antler may be of more functional importance than others (Leung and Forbes, 1996, Swaddle, 2003).

Methods

Study Areas and Red Deer Populations

Animals used in the study were 3120 males, 2–13 years old, harvested during hunting activities in naturally occurring populations of Iberian red deer in southwestern Spain. Data were collected from 1997 to 2006. Antler data correspond to 9 years (springs from 1997 to 2005) according to the moment when the antlers were developed. Data came from three large study areas or metapopulations within the natural distribution area of Iberian red deer in southwestern Spain: Sierra de San Pedro with about 130 000 ha, Monfragüe with approx. 30 000 ha and Sierra Morena of nearly 80 000 ha. In all these areas deer feed mostly on natural pasture and Mediterranean shrub plants (*Phyllirea*, *Arbutus*, *Erica*, *Cistus*, etc.; Rodríguez-Berrocal 1993), although it is quite common that managers provide some supplementary food (hay, alfalfa) during 1 or 2 months between late summer and early autumn (Carranza, 1999, Sánchez-Prieto et al., 2004), which is the limiting season for red deer in this latitude (Carranza et al., 1991, Rodríguez-Berrocal, 1993, Carranza, 1999, Bugalho and Milne, 2003). From October to February, thousands of red deer stags are hunted every year in the Spanish typical commercial hunt called montería. In this type of hunt, packs of dogs are released within a shrub area to move the deer outwards to the sites where hunters are placed. Normally, every male deer of 2 years or more can be legally shot. Hunting pressure on males is regulated basically by allowing only one montería per year in the same area. Because hunters can normally shoot every male above 2 years there is little opportunity for hunting bias to particular males, and montería is the less biased procedure to obtain data from hunted red deer (Martínez et al. 2005). This study never provoked hunters to shoot additional deer (see e.g. Mysterud et al., 2001, Loe et al., 2003, Bonenfant et al., 2003, Carranza et al., 2004 for examples of the use of harvesting data).

Data Recording

Field data

At the end of each hunting day we visited the place where culled animals were gathered. For every animal we recorded a number of measurements in the field and removed mandibles for further measurements in the laboratory. In the field we recorded body weight of the complete carcass between 1 and 3 h after death by using an electronic scale to the nearest 0.5 kg. Males were always weighed before evisceration. For some stags, we had access only to the heads and not the bodies. This was a consequence of carcasses being processed by slaughters in the field simultaneously with our work, so only a random subsample of 1043 males was weighed.

The following measurements for both sides of the antlers were also taken in the field: (1) antler length: length of the main beam from burr to the most distant tip following external shape; (2) length of the brow-tine: the lowest and most prominent branch of the antler; (3) length of the trez-tine: the middle branch of the antler; (4) antler width: we measured perimeters at four points: (a) burr circumference, (b) base circumference, above the burr and below the brow-tine, (c) lower circumference, between the brow-tine and the trez-tine and (d) higher circumference, between the trez-tine and the crown; (5) antler tines: total number of points (tines) ≥2 cm long (Fig. 1).

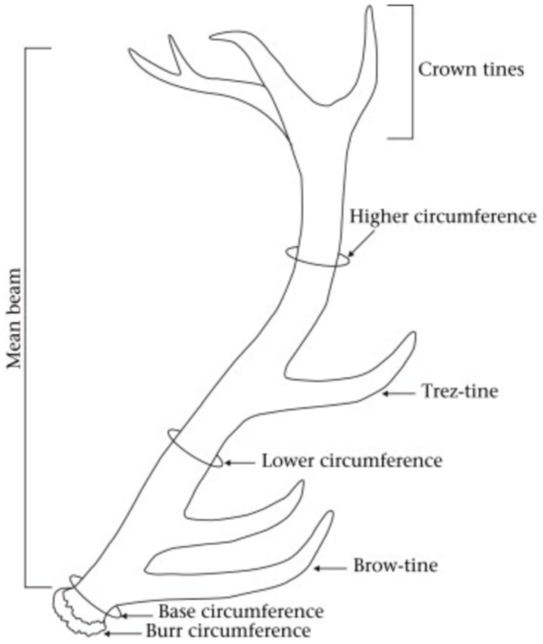


Figure 1. Diagram showing red deer antler features.

It was not possible to take all measurements for all individuals, so sample sizes are different among measurements. Nor was it possible to take replicate measurements in the field, so we took repeated measurements on subsamples of 20 individuals to test for measurement errors (see Appendix).

Laboratory data

Age was estimated by tooth sectioning and counting of cementum growth layers at the interradicular pad under the first molar and checked by noting eruption patterns in younger animals (Mitchell 1967). Ages are expressed in completed years from birth, so an animal aged *n* is living its $n + 1$ year of life, as used for humans.

We also measured jawbone length (mandible size) as the maximum straight line from rearmost point of ramus to the most distal part of the incisor alveoli excluding the incisors.

Subdivisions within study areas

We obtained data from different zones within each study area (Sierra de San Pedro: 15 zones; Monfragüe: six zones; Sierra Morena: 11 zones). A zone is a well-isolated area due to physical characteristics of the habitat that restrict deer movement, such as topography, vegetation, landuse or roads, so that deer can move freely within but difficultly between zones. Some zones corresponded to individual hunting estates that had been surrounded by high (2.1-m) mesh fences that prevented deer from entering or leaving the estate. Other zones, however, consisted of a set of adjacent and nonfenced properties, so that animals could move freely among them. There were not noticeable differences in habitat characteristics or in feeding management practices between fenced and open estates. However, in fenced estates hunting pressure is more effectively controlled by the owner to allow some stags to reach maturity, whereas in nonfenced estates most males are shot quite young. Also, females are more commonly culled in fenced than in unfenced estates. These practices produce big differences in the age structure of the male subpopulation as well as the sex ratio of adults.

Zones were categorized according to physical condition of individuals living there as an indirect measure of environmental disturbance. Body weight depended on age (quadratic relationship) and date of shooting (multiple regression: $R^2 = 0.486$, $F_{3,950} = 299.13$, $P < 0.0001$; date of shooting: coefficient ± SE = 2.609 ± 0.284, $P < 0.0001$; age: 8.669 ± 0.340, $P < 0.0001$; age^2: −1.114 ± 0.075, $P < 0.0001$). The residuals from this regression were used to obtain a measure of male physical condition (adjusted body weight), and zones were divided into two groups according to the average value for these residuals in each zone: good condition zones (positive mean residuals; $N = 767$) and bad condition zones (negative mean residuals; $N = 2030$). This zone division was consistent with that obtained when mandible size, antler size or 2-year-old males' weight were used instead.

An overall antler size variable was obtained by averaging the standard scores of the four antler measures that were recorded for all males (i.e. st. [(Right + Left)/2] of antler length, base circumference, lower circumference and antler tines). Antler size also followed a quadratic relationship with males' age (quadratic regression: $Y = -2.164 + 0.853X - 0.053X^2$, $R^2 = 0.561$, $F_{2,2215} = 1413.41$, $P < 0.0001$) and the residuals (age-adjusted antler size) were significantly related both with adjusted body weight (simple regression: $Y = -0.085 + 0.025X$; $R^2 = 0.299$, $F_{1,737} = 314.11$, $P < 0.0001$) and with adjusted mandible size (simple regression: $Y = -0.074 + 0.289X$, $R^2 = 0.251$, $F_{1,1544} = 516.60$, $P < 0.0001$), showing that bigger and heavier males also had bigger antlers.

Analysis of FA

To obtain the indexes of antler asymmetry we followed Palmer & Strobeck (2003). Higher values in these indexes indicate less symmetric antlers. Details on how we calculated FA are shown in the Appendix.

Using multiple traits

The use of several traits increases the power when testing for heterogeneity of FA among individuals and groups because each trait provides an independent estimate of the underlying developmental instability of an individual (Palmer & Strobeck 2003). Therefore, by using the size-adjusted estimates of FA for the measured antler traits (see Appendix), we finally obtained three independent FA indexes per male: (1) antler beam: a composite FA index including antler length, base circumference and lower circumference because these three measures are different dimensions of the same structure and may not yield independent estimates of DI (Leamy, 1993, Klingenberg and Zaklan, 2000), (2) number of antler tines FA index and (3) length of antler tines: a composite FA index including the lengths of brow-tine and trez-tine.

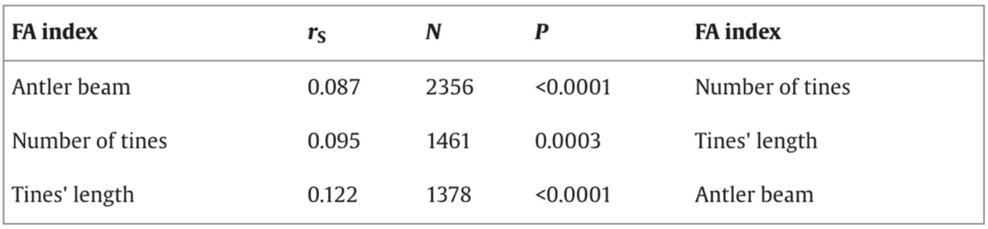
The FA index values for these three traits were used as the dependent variables in subsequent analyses. These variables have truncated normal distributions because they are based on the unsigned values of FA ($|R - L|$). However, the difference between the means of two truncated normal distributions provided a robust and unbiased estimate of the difference between the variances of the untransformed normal distributions (Levene's test for heterogeneity of variance: Levene 1960). This is why these indexes are so useful to test heterogeneity in FA among groups (Palmer & Strobeck 2003).

Testing for differences between individuals, groups and traits

First, as a prior step to the analysis of FA (Palmer & Strobeck 2003), we used the subsample of 20 males for which we took repeated measurements to conduct a mixed model (general linear model procedure: GLM) on repeated measurements of $|\ln R - \ln L|$ to determine whether FA varied among individuals. Traits were introduced as fixed factor and individuals as random factor.

Second, with all the data, we carried out another analysis to test for differences between groups and traits. The FA values of the three traits obtained per male, with different sample sizes in each, were correlated among them (see Table 1). Therefore, as suggested by Van Dongen et al. (1999), we chose a repeated-measures design and used a linear mixed model procedure fitted by restricted maximum likelihood (REML: SPSS software vs 13; SPSS Inc., Chicago, IL, U.S.A.), which allows one to model data with correlated and nonconstant variability. Data from different traits within single individuals were treated as repeated measures. We chose the ‘unstructured’ covariance structure for the residuals because there was nonconstant variance within traits while the covariances between them were significant. The model including all main effects showed the best-fitting values of the information criteria (−2 restricted log likelihood and Akaike's information criterion). Fixed effects were zone condition (bad or good condition: see above), management (open or fenced estates), study area, age, trait and year. Then another model was constructed by first adding all meaningful two- and three-way interactions and second sequentially dropping and re-adding individual terms until all interactions included in the model were significant. We avoided those interactions with missing cells because we had to use the type III sum of squares method to assess the significance of fixed terms. Nevertheless, during the stepwise procedure, changes in deviance between subsequent steps were assessed.

Table 1. Correlations between FA across traits



FA index	r_S	N	P	FA index
Antler beam	0.087	2356	<0.0001	Number of tines
Number of tines	0.095	1461	0.0003	Tines' length
Tines' length	0.122	1378	<0.0001	Antler beam

Spearmen rank correlation and sample size given in columns correspond to the relationship between each trait pair placed on the same row.

Model results are presented for all main effects and significant interactions. The significance of parameter estimates was assessed with t tests. In the figures we present mean predicted values from the model.

Results

Differences Between Individuals

The GLM analysis on repeated measurements of $|\ln R - \ln L|$ showed that both individuals and traits contributed significantly to explain the variance in FA (individuals: $F_{19,99} = 4.151$, $P < 0.001$, partial eta squared = 0.443; traits: $F_{1,99} = 81.862$, $P < 0.001$, partial eta squared = 0.453).

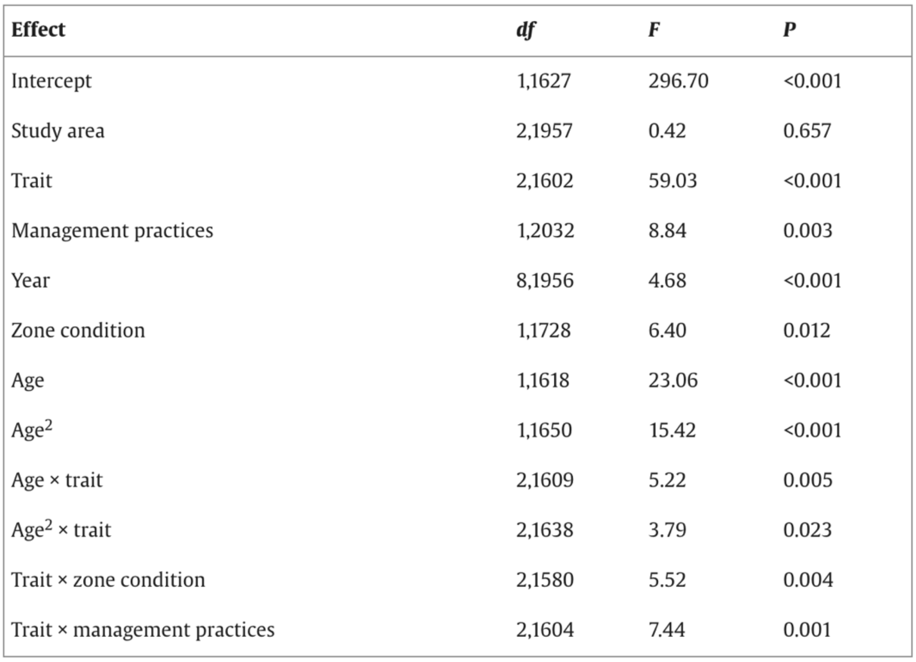
The three indexes of antler FA were positively correlated between them (Table 1), but they were negatively correlated with their corresponding trait size (the average value of the standard scores for the antler variables included in each FA index). Larger antlers were more symmetric, and the relationship was stronger for the most asymmetric trait, the length of antler tines (corrected for ties Spearman rank correlation: $r_S = -0.168$, $N = 2369$, $P < 0.0001$ for antler beam; $r_S = -0.152$, $N = 2692$, $P < 0.0001$ for number of tines; $r_S = -0.332$, $N = 1480$, $P < 0.0001$ for length of antler tines). Although correlation coefficients were not very high, the negative relationship was maintained when antler size was controlled by age (Spearman rank correlation between age-adjusted antler size and antler FA: $r_S = -0.101$, $N = 2218$, $P = 0.005$). In this case, antler FA is a composite index of FA obtained with the same variables included when computing the age-adjusted antler size variable (see Methods).

Finally, a slight but significant negative relationship was also found between the body weight (controlling by age; see Methods) and the composite index of antler asymmetry (Spearman rank correlation between adjusted body weight and antler FA: $r_S = -0.075$, $N = 739$, $P = 0.041$). However, there was no significant relationship when mandible size was considered instead of body weight (Spearman rank correlation between adjusted mandible size and antler FA: $r_S = -0.029$, $N = 1546$, $P = 0.255$).

Differences Between Groups and Traits

Results from mixed model analysis (REML) are shown in Table 2. The three antler traits considered in the analysis showed significant differences in their FA indexes. The length of antler tines (trez- and brow-tines) was a more asymmetric trait than antler beam (parameter estimate for the difference between antler beam FA index and tines' length FA index: mean ± SE = -1.4004 ± 0.018, $t_{1294} = -7.751$, $P < 0.0001$) and number of tines (parameter estimate for the difference between number of tines FA and tines' length FA: mean ± SE = -0.0485 ± 0.024, $t_{1552} = -2.020$, $P = 0.044$). This result was consistent for the three study areas (Fig. 2).

Table 2. Effects influencing variability in antler FA (standardized FA indices of three antlers traits: antler beam, number of tines and length of tines)



Effect	***df***	***F***	***P***
Intercept	1,1627	296.70	<0.001
Study area	2,1957	0.42	0.657
Trait	2,1602	59.03	<0.001
Management practices	1,2032	8.84	0.003
Year	8,1956	4.68	<0.001
Zone condition	1,1728	6.40	0.012
Age	1,1618	23.06	<0.001
Age^2	1,1650	15.42	<0.001
Age × trait	2,1609	5.22	0.005
Age^2 × trait	2,1638	3.79	0.023
Trait × zone condition	2,1580	5.52	0.004
Trait × management practices	2,1604	7.44	0.001

Results from a mixed model (REML). Only main effects and significant ($P<0.005$) interaction terms are shown. Zone condition refers to a variable with two levels: good and bad condition. FA data from different traits within single individuals were treated as repeated measures.

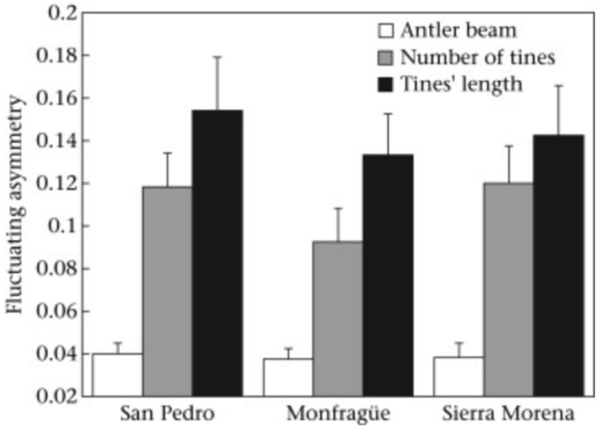


Figure 2. Standardized FA indices of three antler traits (antler beam, number of tines and length of tines) in three study areas. Bars show predicted values from the model (mean ± SE; Table 2) with age set to its mean value. Number of males (*N*) for San Pedro, Monfragüe and Sierra Morena: 1419, 822 and 321, respectively.

In the three study areas, males from zones in good condition (see Methods) showed more symmetric antlers than males from zones in bad condition (parameter estimate: good minus bad condition zones:
mean ± SE = −0.029 ± 0.008, $t_{1325} = -3.617$, $P < 0.001$; Fig. 3a). The interaction between zone condition and trait terms was significant (Table 2), indicating that tines'

length was not only the most asymmetric antler trait but also the most sensitive to differences in environmental conditions (Fig. 3b).

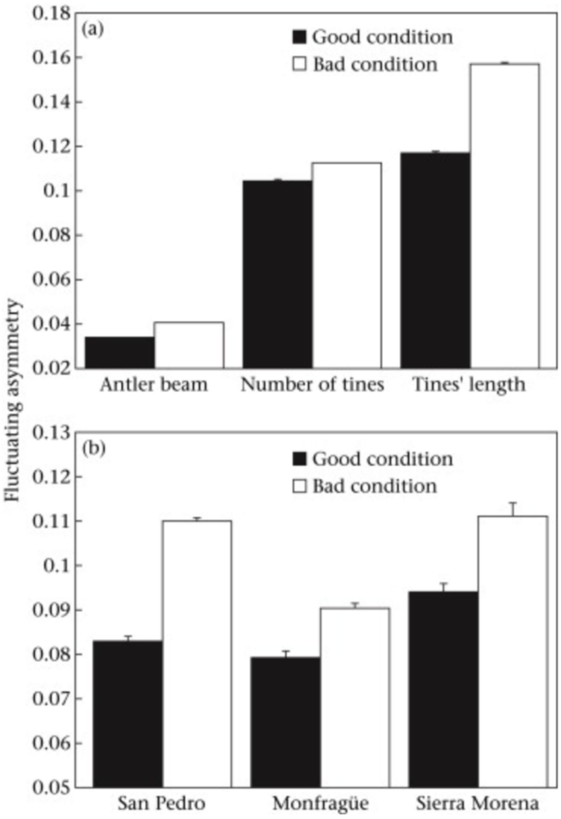


Figure 3. Effect of environmental conditions (good and bad condition zones) on FA. (a) Effect on antler' traits. (b) Effect on the three study areas. Bars show predicted values from the model (mean ± SE; Table 2) with age set to its mean value.

Management practices also had significant effects on antler asymmetry. For the three study areas, males living in open estates had more asymmetric antlers than males living in fenced estates (parameter estimate: open minus fenced estates: mean ± SE = 0.018 ± 0.008, $t_{1383} = 2.184$, $P = 0.029$; Fig. 4a), and this effect was again stronger for the most asymmetric traits (Table 2, Fig. 4b). Nevertheless, under both types of management practices, males from zones in good condition had more symmetric antlers than males from zones in bad condition (mean difference in predicted values from the model ± SE: open estates: good minus bad condition zones: −0.011 ± 0.002; $t_{4429} = -4.74$, $P < 0.0001$; fenced estates: good minus bad condition zones: −0.009 ± 0.001, $t_{3253} = -6.50$, $P < 0.0001$; Fig. 5).

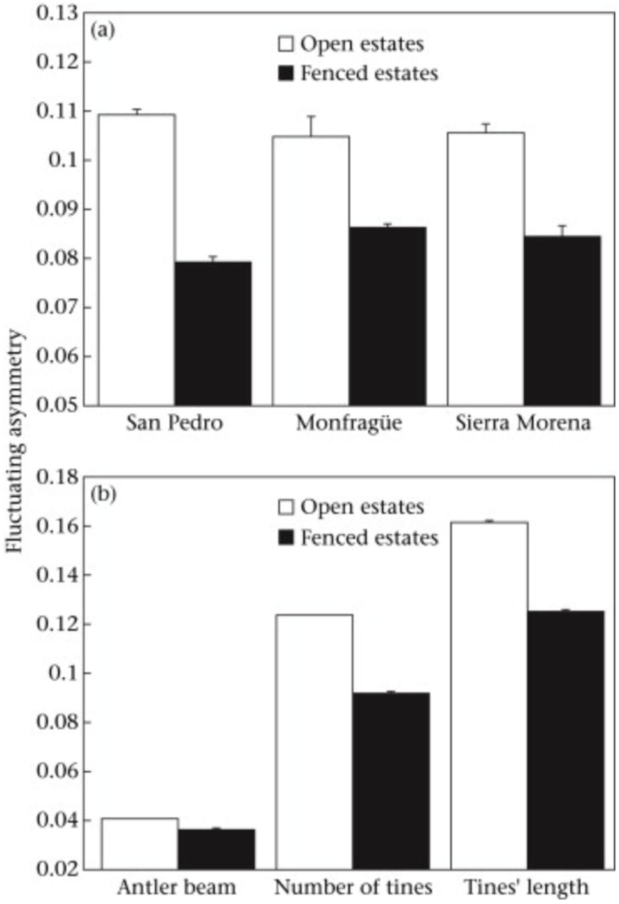


Figure 4. Effect of management practices (open and fenced estates) on FA. (a) Effect on the three study areas. (b) Effect on antler' traits. Bars show predicted values from the model (mean ± SE; Table 2) with age set to its mean value.

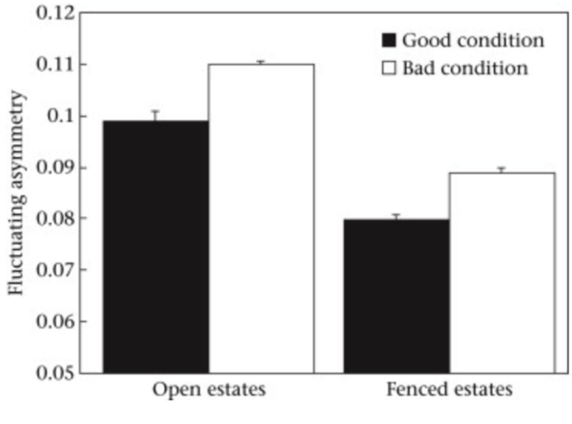


Figure 5. Effect of management practices (open and fenced estates) and environmental conditions (good and bad condition zones) on FA. Bars show predicted values from the model (mean ± SE; Table 2) with age set to its mean value.

The relationship between age and FA was U-shaped, with mature males (from 5 to 8 years old) bearing the most symmetric antlers (parameter estimate:

slope ± SE = −0.0245 ± 0.0074, $t_{1295} = -3.291$, $P = 0.001$ for age; slope ± SE = 0.0216 ± 0.0007, $t_{1294} = 2.963$, $P = 0.003$ for age^2; Fig. 6). The interaction terms age × trait and age^2 × trait were significant (see Fig. 7a), with the U-shaped relationship more conspicuous for the most asymmetric traits. However, there were no differences between study areas, management practices or environmental conditions (see Fig. 7b–d).

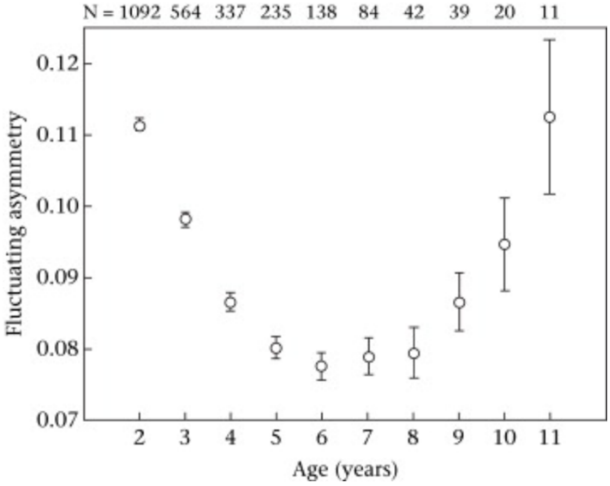


Figure 6. U-shaped relationship between age and antler FA. Fluctuating asymmetry refers to predicted values from the model (mean ± SE; Table 2). Individuals were introduced in the model as subjects and traits as repeated measurements per male. Number of males for each age group is shown above the graph.

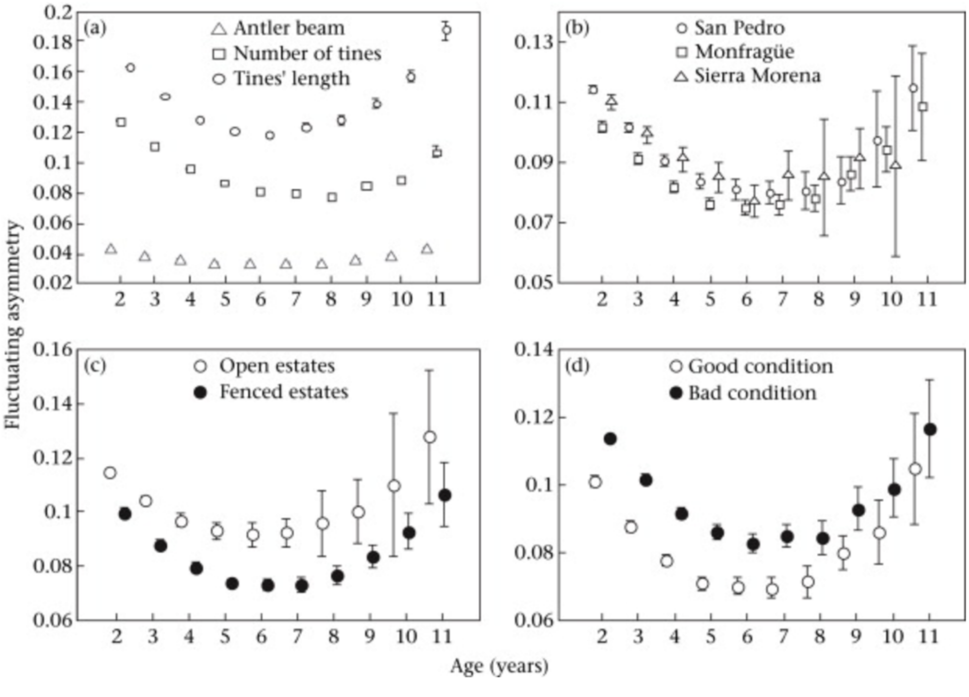


Figure 7. Effects on the relationship between age and antler FA of (a) antler' traits, (b) study areas, (c) management practices (open and fenced estates), (d) environmental

conditions (good and bad condition zones). Fluctuating asymmetry refers to predicted values from the model (mean ± SE; Table 2).

Finally, we found a significant effect of year on antler FA (Table 2). For all study areas and conditions, antlers were more asymmetric in 1999 (parameter estimate ± SE = 0.009 ± 0.003, $t_{1972} = 2.979$, $P = 0.003$, reference: 2005).

Discussion

All antler traits here considered showed fluctuating asymmetry, although the level of asymmetry and the response to environmental disturbance seemed to be trait specific (Leung & Forbes 1996). Antler beam (including antler length, base circumference and lower circumference) was the most symmetrical trait, while the number of tines and mainly tines' length (including brow-tine and trez-tine) showed higher heterogeneity in FA.

The results supported the general assumption of the FA hypothesis, according to which asymmetry of bilateral sexual traits may reflect developmental instability at different magnitudes of environmental stress. This statement is based upon the following findings.

(1) There was a positive correlation between the unsigned FA of the three antler traits. This is an important premise of the FA hypothesis since, as Houle (1998) pointed out, ‘if there is no correlation, and the asymmetry of one trait does not even predict asymmetry of other traits, it would be difficult to see how asymmetry can predict anything else’ (see also Whitlock, 1996, Palmer and Strobeck, 2003). Still, the among-trait correlations found here were weak (and nonsignificant in other studies: Kruuk et al., 2003, Bartoš and Bahbouh, 2006). A suggested explanation is that the environment may be not constant throughout antler development. This would result in different levels of asymmetry if independent parts of the antler developed at different times (Swaddle 2003).

(2) Individual differences in antler FA related to age-corrected body mass and antler size. Thus, heavier stags had not only larger antlers (Alarcos 2007) but also more symmetric antlers. As far as we know this is the first study to show a significant relationship between antler FA and some male characteristics associated with fitness in red deer (Clutton-Brock et al., 1982, Kruuk et al., 2002, Malo et al., 2005, Bartoš and Bahbouh, 2006).

A negative relationship between weapon size and asymmetry (another assumption of the FA hypothesis: Møller, 1990, Møller and Pomiankowski, 1993, Markusson and Folstad, 1997, Ditchkoff et al., 2001) was also reported by Bartoš & Bahbouh (2006) for red deer antlers. By contrast, Kruuk et al. (2003) found no relationship between relative FA and antler size for red deer on Rhum. However, after controlling for age, they also found a significant negative association between the brow-tine length and its relative asymmetry, this trait being the tract for which the negative correlation between asymmetry and size was higher both in Bartoš & Bahbouh (2006) and in this study.

(3) Frequency distribution of signed asymmetry displayed leptokurtosis, as predicted if asymmetry reflected differences in developmental precision among groups or individuals (Houle, 1997, Gangestad and Thornhill, 1998). Our data belong to a heterogeneous sample of groups with different degrees of antler asymmetry according to males' age, year and study area. During 1999, antlers showed higher asymmetry

probably related to the drought period that took place in 1998 and 1999 in southern regions of Spain (Alarcos 2007). Furthermore, regardless of males' age, individuals living in zones that are likely to be in worse conditions (see Methods) had more asymmetric antler traits; and the same can be said for males from open estates with respect to those from fenced estates. This finding is consistent with the finding that males in open estates also had less body mass and smaller antlers than those in fenced estates (Alarcos 2007). Differences in management practices in the two types of estates may explain these results. In particular, open estates maintain highly female-skewed adult sex ratios (from three to six females per male: Martínez et al. 2002) due to intense hunting pressure on males (Carranza 1999), and the presence of high female numbers is known to hamper the development of both body size and antler size in red deer (Clutton-Brock et al., 1982, Clutton-Brock and Albon, 1989).

The debate on the adaptive significance of FA has focused mainly on whether there is a direct association between trait asymmetry and fitness or whether trait asymmetry is just a correlate of male general quality because of its relationship with male condition (or trait size). However, it is not always possible to distinguish between direct and indirect association with fitness (Swaddle 2003) because the degree of asymmetry and its response to stress may depend on the functional importance of the trait (Leung and Forbes, 1996, Swaddle, 2003) and comparisons between traits of different functional importance have only rarely been made (Van Dongen 2006). When asymmetry represents a functional problem for a trait, with a direct and negative effect on fitness, selection should favour compensation mechanisms to maintain the developmental stability and symmetry of the trait (Palmer and Strobeck, 1986, Watson and Thornhill, 1994, Clarke, 1998b). In that case, it is not necessary to assume that symmetry must have a special value as a signal (Borges 2000).

Antlers are primarily weapons used in strong and overt fighting (Clutton-Brock 1982). Winning fights has paramount importance in male reproductive success in red deer (Clutton-Brock et al., 1982, Clutton-Brock et al., 1988). Mature stags fight by opposing their antlers, so that the right antler of one male pushes against the left antler of his opponent and vice versa. Antler tines function to prevent the antler of the rival male from sliding along the beam and reaching the head or eyes. This use of antlers during fighting may represent a selective force for a higher symmetry in antler beam and in the number of tines (as has been pointed out by other authors; Kruuk et al. 2003). Also, antlers mass should depend on the number of tines and antler beam dimensions (Alarcos 2007). Thus, merely for functional reasons, it is sensible to assume that such a heavy weapon must not be very unbalanced, in particular during the ages of maximum development of the trait. Asymmetry in brow- and trez-tine lengths, on the other hand, may have less functional importance if the main use of tines is to prevent the antler of the rival male from sliding (rather than to puncture him while opposing his corresponding tine), allowing for a higher heterogeneity in the FA of these traits.

Red deer antlers are subjected to directional selection (Kruuk et al. 2002), which may reduce the effectiveness of developmental control mechanisms (Møller & Pomiankowski 1993). Consequently, there are possibilities especially for those traits with less functional role in fighting to be condition-dependent signals: values for tines' length varied in a wide range (asymmetry needed to be large enough to be detected: Swaddle 2003) and the relationship between FA and trait size was also stronger than the same relationship for the other characters of the antler (Bartoš & Bahbouh 2006; this study).

The results obtained here also confirm that, in the red deer, antler asymmetry is an age-dependent variable. Therefore, it is necessary for FA studies to control for age-related changes in both the size and the asymmetry of antlers, as Kruuk et al. (2003) had already suggested. In agreement with previous studies using transversal data (Baccus and Welch, 1983, Smith et al., 1983, Pélabon and Van Breukelen, 1998, Solberg and Sæther, 1993, Putman et al., 2000, Ditchkoff et al., 2001; see also Møller, 1997, Møller and Nielsen, 1997), our results also show an inverse relationship between the degree of asymmetry and the age of the individual, at least until the end of reproductive age. Three explanations have been given for a positive relationship between symmetry and age: (1) the more symmetrical antlers in adult and older males may be a result of viability selection (including both hunting and natural selection) in younger age classes (Solberg and Sæther, 1993, Ditchkoff et al., 2001), (2) animals may became less sensitive to environmental stress as they age (Putman et al. 2000) or (3) antler asymmetry is not likely to be a priority for younger adults, who would instead be more interested in investing resources to develop full adult size quickly (Hayden et al. 1994). Also, antler development in red deer is completed only later in life (by 6 years of age in our populations, unpublished data), and a reduction of asymmetry at the end of the growth period may be expected because more stability in development should be achieved as growth progresses (compensational growth hypothesis: Swaddle & Witter 1997; but see also the model by Aparicio 2001 for the effect of growth on the relationship between trait size and FA).

However, our finding was not a progressive reduction of asymmetry with age but a U-shaped relationship between age and antler asymmetry, inversely related to the relationship between antler size and age (Alarcos 2007). The increase of antler asymmetry for older stags had not been reported until now, and the previous explanations cannot account for it. Antlers of senile males may be more asymmetric as a direct effect of food stress as a consequence of the relationship between males' tooth wear and senescence (Carranza et al., 2004, Carranza et al., in press) because this process in our red deer population also causes a loss of body mass and a reduced antler size. However, it also might be that, later in life, the development of renewable structures becomes less precise owing to accumulated accidents in the morphogenesis as one of the effects of senescence (Stige et al. 2006). Furthermore, selection acting on mechanisms that control developmental stability yield fitness returns that are more likely during prime age so, as in other processes related to senescence (Hamilton 1966), any imprecision in those mechanisms in later age are less likely to be counterselected.

Therefore, we suggest an effect of sexual selection on the precision of antler development during prime age, in agreement with the idea suggested by Hayden et al. (1994), because this sexual character evolved primarily as a weapon to fight against males (Clutton-Brock 1982), especially during the years with the highest reproductive chances (Clutton-Brock et al., 1982, Clutton-Brock et al., 1988, Clutton-Brock and Albon, 1989). Longitudinal studies may be crucial to test this hypothesis. The longitudinal study by Kruuk et al. (2003) did not find any significant relationship between age and relative antler FA, although their results showed a very high sampling error. Our estimation for this relationship lay within the confidence interval reported by them (but see also a recent work by Bartoš et al. 2007).

Finally, it is interesting to note that, although the results support several assumptions of the FA hypothesis, the relationship between symmetry and fitness components found here was weak, as predicted by Clarke (1998a) (see also Stenström 1998 and Kruuk et al. 2003). This endorses the notion that very large samples are needed to detect stress effects on FA, which makes studies of fluctuating asymmetry an uneasy and expensive procedure to assess environmental stress (Bjorksten et al. 2000). On the contrary, it is well documented that antler size itself is very sensitive to stress and that it is a good indicator of males' condition as well as a reliable predictor of males' reproductive success (review in Bjorksten et al., 2000, Pélabon and Joly, 2000, Malo et al., 2005, Bartoš and Bahbouh, 2006; but see Møller 2000 and Van Dongen & Lens 2000). Nevertheless, as pointed out by Van Dongen and Lens (2000), given the observed heterogeneity in FA stress, conservational studies might consider combining values from FA and trait size as a more sensitive method to detect stress.

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Appendix: Obtaining FA Indexes

To obtain our indexes of antler asymmetry we took the following steps from the protocol by Palmer & Strobeck (2003):

Validating the data

We carefully checked the data file to (1) remove all cases with broken or worn antler tines or branches, (2) detect outliers and data errors (recording, transcription and data entry errors) by visual inspection of scatter plots (scatter plots of left versus right measurements for each trait (measurement error (ME) outliers detection) and scatter plots of $(R - L)$ for one trait versus $(R - L)$ for another related trait (FA outliers detection), (3) correct transcription and data entry errors in the data file and (4) remove outliers by using the Grubb's test and the sequential Bonferroni correction.

Testing FA relative to measurement error

Our study did not allow us to take replicate measurements of all traits in all males, so we took repeated measurements on two subsamples of individuals: a subset of 20 males measured three times by the same person (ideal condition) and a subset of 10 males measured three times by different people (the worst condition). All traits were measured except number of tines, for which there is not measurement error (Kruuk et al. 2003).

We estimated the repeatability of FA (intraclass correlation coefficient of signed FA) and conducted a procedure to test for FA relative to ME.

FA and ME values were estimated using a one-way ANOVA on signed asymmetry, as proposed by David et al. (1999), which allows the computation of mean squares between individuals ($\mathrm{MS}_{\mathrm{SA}}$) and within individuals ($\mathrm{MS}_{\mathrm{err}}$). In this model, the estimate of FA is $\mathrm{FA} = (\mathrm{MS}_{\mathrm{SA}} - \mathrm{MS}_{\mathrm{err}})/2r$, where r is the number of replicate measurements, and the estimate of ME is $\mathrm{ME} = \mathrm{MS}_{\mathrm{err}}/2$. Nevertheless, the two-way, mixed-model ANOVA (with trait sides as fixed factor and individuals as random factor) procedure proposed by Palmer and Strobeck, 1986, Palmer and Strobeck, 2003 yielded similar results.

Two antler measures (burr circumference and higher circumference) showed a high value of ME relative to FA when they were measured in the worse condition (different people). Irregular shape of burr circumference and variations in the chosen point to measure higher circumference (between the trez-tine and the crow) may explain the high error in these measures. Thus, they were removed in subsequent analyses of antler FA (Table A1).

Calculating standardized FA indexes

We tested for size dependence of FA using nonparametric tests of association (Spearman rank correlation coefficients) between trait unsigned asymmetry $|R - L|$ and trait size $(R + L)/2$. Unsigned asymmetry was either independent or increased with trait size (Table A2), so we used size-adjusted indices of FA as proposed by Palmer & Strobeck (2003). Size-adjusted estimates of FA were quantified as $|\ln R - \ln L|$, which are numerically equivalent to conventional relative asymmetry indices, estimated by dividing absolute FA by the average of both sides, but avoiding the problem that numerator and denominator are not independent.

Departures from ideal FA

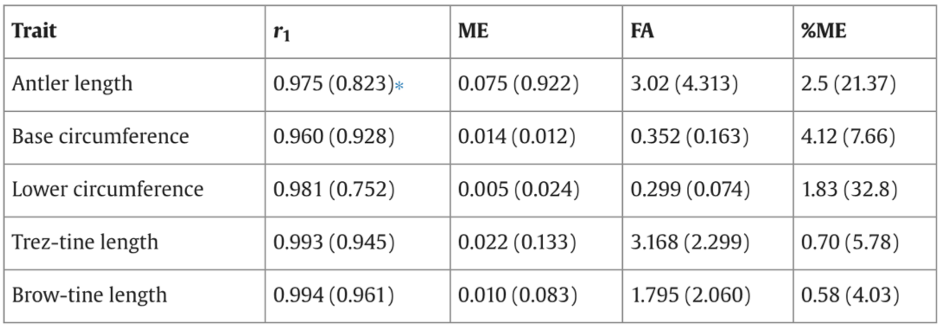
Testing for directional asymmetry (DA)

Tests for DA (when one side is consistently larger than the other side in the same direction) were conducted for all traits using the conventional one-sample t tests of mean ($\ln R - \ln L$) versus zero. Antler length showed a very slight DA in favour of the right side, which became statistically significant due to the large sample size (mean ± SE = -0.002 ± 0.001, $t_{2588} = -2.47$, $P = 0.013$). However, as this mean value was much lower than the average deviation around the mean ($0.789\mathrm{var}(\mathrm{lnR} - \mathrm{lnL}) = 0.035$), asymmetry in antler length can be considered fluctuating and is included in FA analyses (Palmer & Strobeck 2003).

Testing for normality in FA

Frequency distribution of signed asymmetry showed a clear leptokurtosis for all antler traits. Values of kurtosis ranged from 3.29 to 11.58, which are much larger than the critical value for testing the significance of kurtosis (Palmer & Strobeck 2003). After removing data file errors and controlling for measurement errors we can assume that the main cause of leptokurtosis is the existence of within-sample heterogeneity in FA. Heterogeneity in FA may be partly due to size-dependent variability, but we had already eliminated this source of heterogeneity by using size-adjusted FA. On the other hand, Van Dongen (1998) and Gangestad & Thornhill (1999) showed that a mixture of samples and individuals with real differences in developmental instability may also produce leptokurtosis (but see Graham et al. 2003). This type of heterogeneity is not a problem for FA studies but rather a required feature to allow the study of the relationships between individual asymmetry and correlated factors (Palmer & Strobeck 2003).

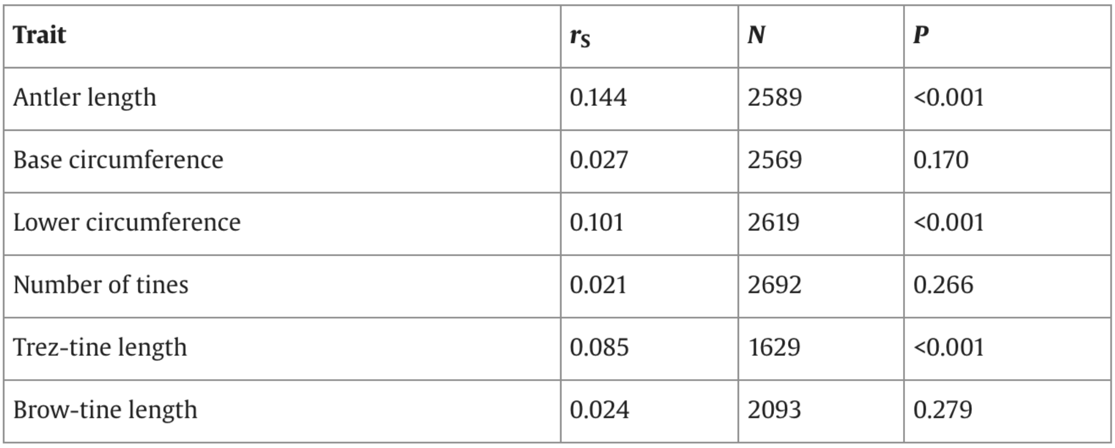
Table A1. Repeatability of FA (intraclass correlation coefficient: r_1) and measurement error (ME) relative to the estimation of FA for each antler trait



Trait	r_1	ME	FA	%ME
Antler length	0.975 (0.823)*	0.075 (0.922)	3.02 (4.313)	2.5 (21.37)
Base circumference	0.960 (0.928)	0.014 (0.012)	0.352 (0.163)	4.12 (7.66)
Lower circumference	0.981 (0.752)	0.005 (0.024)	0.299 (0.074)	1.83 (32.8)
Trez-tine length	0.993 (0.945)	0.022 (0.133)	3.168 (2.299)	0.70 (5.78)
Brow-tine length	0.994 (0.961)	0.010 (0.083)	1.795 (2.060)	0.58 (4.03)

* Data obtained under the worse measurement conditions (different people).

Table A2. Relationship between size and FA



Trait	r_S	N	P
Antler length	0.144	2589	<0.001
Base circumference	0.027	2569	0.170
Lower circumference	0.101	2619	<0.001
Number of tines	0.021	2692	0.266
Trez-tine length	0.085	1629	<0.001
Brow-tine length	0.024	2093	0.279

Spearman coefficients of rank correlation between trait unsigned asymmetry $|R - L|$ and trait size $[(R + L)/2]$.