

# Fluctuating asymmetry in a secondary sexual trait: no associations with individual fitness, environmental stress or inbreeding, and no heritability

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## Abstract

It has been suggested that fluctuating asymmetry (FA) in secondary sexual traits may be a useful indicator of either individual quality or environmental stress. We tested this concept using a series of analyses of FA in male antler size in a wild red deer (*Cervus elaphus*) population, using four measures of size repeated across successive years on the same individuals. We found no consistent evidence of correlations between traits in levels of FA, nor of any associations between known environmental or developmental conditions. None of the four measures of FA showed a significant heritability (average  $h^2 = 0.041$ ), nor was there any evidence of inbreeding depression. For three of the four traits, fluctuating asymmetry did not predict either annual or lifetime breeding success. However there were significant associations between breeding success and FA in antler length. Given the series of null results in our other tests, it seems likely that this was a direct mechanistic effect rather than because measures of FA were indicative of individual quality or condition.

## Introduction

It has been suggested that bilateral symmetry in morphometric traits reflects developmental stability, and that symmetrical individuals should therefore have quantifiable advantages over their asymmetric counterparts. By this reasoning, any subtle random deviations from perfect bilateral symmetry, known as fluctuating asymmetry (FA), can be used as an indicator of individual quality, and in particular of the genetic or environmental stresses to which an individual may have been subject during development (Møller & Pomiankowski, 1993; Møller & Swaddle, 1997; Thornhill & Møller, 1998). The logical appeal of this idea, and the ease with which FA can be quantified in large numbers of individuals, has generated studies of FA in a wide range of biological systems, and research in the area has been further encouraged by

explorations of the statistical properties of asymmetry measures (e.g. Whitlock, 1996; van Dongen, 1998).

Because secondary sexual traits frequently show condition-dependent expression, with only individuals of superior condition being able to sustain the cost of substantial trait exaggeration, they are obvious candidates for indicators of either genetic quality or environmental stress (Andersson, 1994). It has further been proposed that asymmetry in sexual traits will be a more sensitive indicator than either their overall size or the asymmetry of nonsexual traits (Thornhill & Møller, 1998). Particular attention has focused on the potential existence of associations between developmental stability, and hence symmetry, in secondary sexual traits and mating success, and several studies have reported associations between FA and male mating success (reviewed in Møller & Swaddle, 1997; Møller & Thornhill, 1998).

However, enthusiasm for the use of FA has been dampened by concerns both of possible flaws in certain key reviews and of a reporting bias in the literature, such that there is currently little consensus on the informativeness of FA (e.g. Houle, 1998; Palmer, 1999;

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Bjorksten *et al.*, 2000). In relation to secondary sexual characteristics, recent results from experimental studies have been less conclusive than earlier examples (e.g. see review in Bjorksten *et al.*, 2000). As an example of the variability in conclusions in the literature on FA, Markow & Ricker (1992) report a positive association between FA and mating success in *Drosophila pseudoobscura* and a negative association in *D. simulans*, whereas Markow *et al.* (1996) find no associations between FA and mating success in either species, and, in an intensive investigation of the effects of environmental stress on FA, Woods *et al.* (2002) also find no evidence of effects of FA on fecundity in *D. melanogaster*. Similarly, Harvey & Walsh (1993) report positive correlations between FA and male mating success in one species of damselfly, whereas Carchini *et al.* (2000), considering a different species of damselfly, find that such effects disappear if body size is also taken into account. Thus a consensus as to the generality of such patterns remains elusive, and can only depend on the accumulation of replicable results from investigations of a range of systems.

The existence of associations between fitness (or condition) and FA in a sexually selected characteristic generates a series of testable predictions:

- 1 If developmental stability is reflected in the FA of a sexual character, this should apply across different aspects of the character, so FA should be correlated across alternative measurements within an individual (Whitlock, 1996).
- 2 Because only high quality individuals can sustain the cost of exaggerated size in a condition-dependent sexually selected trait, there should be a negative association between FA and trait size (Møller, 1980).
- 3 Fluctuating asymmetry should reflect environmental stress, such that it increases when environmental conditions are harsh either during the development of the trait or during early development of the individual (Hoffmann & Parsons, 1991; Parsons, 1992).
- 4 In populations subject to inbreeding depression, inbred individuals should show higher levels of FA than their less inbred counterparts (Lerner, 1954; Soulé, 1982).
- 5 If FA reflects developmental stability and developmental stability reflects individual quality, then FA should be correlated with an individual's fitness: more symmetrical individuals should have higher breeding success, both in a given breeding attempt and across their entire lifespan (Møller & Swaddle, 1997; Møller & Thornhill, 1998; Thornhill & Møller, 1998).
- 6 Finally, given the high levels of genetic variation underlying secondary sexual traits (Pomiankowski & Møller, 1995), as well as the increasing evidence for a heritable genetic basis to condition, and underlying any explanation as to why in certain biological systems females should choose more symmetrical mates, we might also expect FA of a secondary sexual trait to be heritable (Møller & Thornhill, 1997).

Because of the difficulties inherent in measuring the necessary variables in the wild, tests of the above hypotheses have generally relied on data from experimental manipulations and/or captive populations. As a result we still know little about the associations between FA and fitness in natural populations: FA in a morphological trait may be relatively easy to measure for a wild population, but breeding success, levels of inbreeding and heritabilities are not. Furthermore, if associations with FA are subtle, any underlying signals may be swamped by environmental variation, requiring large sample sizes and long-term data for statistical power. Thus, to our knowledge, the full set of the above hypotheses has not been simultaneously tested on a natural population.

The majority of studies to date have concentrated on the scenario in which sexual selection is driven by female choice. Here, we consider an alternative situation in which sexual selection operates directly through intra-sexual conflict. We analysed asymmetry in red deer (*Cervus elaphus*) antlers, which are used by males as weapons in fights over access to females. Antler size is positively correlated with mating success (even after correcting for body size, Kruuk *et al.*, 2002), and is therefore under sexual selection, but there is no evidence that antlers are the subject of female choice (Clutton-Brock, 1982). Results from previous studies of FA in weapons in other ungulate species have reached mixed conclusions, possibly because sample sizes are frequently small (Palmer, 1999). For example, Lagesen & Folstad (1998) found significant associations between FA and levels of immune activity in semidomesticated reindeer (*Rangifer tarandus tarandus*), but contrasting directions in the estimates for specific immune parameters make it difficult to interpret the biological meaning of the results. Malyon & Healy (1994) report a negative association between measures of FA and dominance rank in eight farmed fallow deer (*Dama dama*), but Pélabon & Joly (2000) found no associations between levels of asymmetry and dominance rank in a sample of 45 male fallow deer in a zoo population. In moose (*Alces alces*), FA in the number of antler points in culled animals increased with antler size, but there was a negative relationship between FA and the carcass weight of an individual after correcting for antler size: for a given antler size, heavier individuals had more symmetrical antlers (Solberg & Saether, 1993). This relationship changed with age classes, highlighting the need to be able to control for age-related changes in both the size and the FA of antlers. Bowyer *et al.* (2001) found a negative relationship between relative FA and antler size in moose, but could not distinguish whether this was because of antler breakage or differences in developmental stability. Similarly, within age classes, asymmetry in roe deer (*Capreolus capreolus*) antlers decreased with size, and increased with population density (Pélabon & van Breukelen, 1998). In gemsbok (*Oryx gazella*), horn asymmetry decreased with a

range of observations of phenotypic quality (Møller *et al.*, 1996). In contrast, in mountain goats (*Oreamnos americanus*), asymmetry in horn size did not reflect individual quality in males (Côté & Festa-Bianchet 2001; although note that there were associations in females), whereas in bighorn sheep (*Ovis canadensis*) there was no association between horn size and multilocus heterozygosity (Fitzsimmons *et al.*, 1995). However we know of no studies to date which have tested for direct associations between FA in male weaponry in ungulates and mating success.

In this paper, we use records of individual lifetime breeding success, a multigenerational pedigree, extensive genotypic data and measures of FA in the secondary sexual trait of male antlers, from a long-term study of the unmanaged red deer (*C. elaphus*) population on the Isle of Rum, Scotland to test the above predictions regarding FA and fitness. We have information on the breeding success of individuals not only in a given year but also over their entire lifespan, so that we can explicitly test for associations between lifetime breeding success (fitness) and asymmetry. We also test for covariance between environmental conditions and levels of asymmetry across different years: biotic (specifically population density) and abiotic (specifically climatic conditions) have wide-ranging and varied effects on almost all aspects of deer life histories and on antler size (e.g. Albon *et al.*, 1987; Coulson *et al.*, 1997; Rose *et al.*, 1998; Kruuk *et al.*, 1999a; Schmidt *et al.*, 2001). Rates of inbreeding in the Rum population have been shown to affect birth weight, juvenile survival and even adult breeding success (Slate *et al.*, 2000, in preparation). Here, we test whether levels of FA reflect inbreeding by testing for associations between FA and multilocus heterozygosity. Finally, knowledge of pedigree relationships also permits estimation of the heritability of FA, to give an indication of the proportion of variation under genetic, rather than environmental, control.

## Materials and methods

### Study population

Data were collected on the unmanaged red deer (*C. elaphus*) population in the North Block of the Isle of Rum, Inner Hebrides, Scotland, between 1974 and 1998. All animals in the study area were known individually, either by natural variation or artificial marks, and were monitored in five censuses carried out each month. Culling of the population ceased in 1973, resulting in an almost three-fold increase in the number of adult females, but a decrease in the number of adult males; since 1982 the population has remained relatively stable around a mean value of 161.6 females ( $\pm 16.1$  SD) and 109.3 males ( $\pm 16.9$  SD). The majority of calves were caught soon after birth, and a blood sample taken for genetic analysis; other individuals were sampled by immobilisation or at post mortem. All sampled deer were genotyped at up to

nine highly variable microsatellite markers (Coulson *et al.*, 1998; Marshall *et al.*, 1998). Further details of the study area and population are given in Clutton-Brock *et al.* (1982).

### Antler measurements

Male red deer grow a new set of antlers each year, after casting the old set between March and May. This facilitates comparison of differences in asymmetry (i) between years, as a result of variance in environmental conditions and (ii) between individuals. Because a stag's antler shape is unique, cast antlers can be assigned to known individuals: DNA analysis has confirmed that assignment is correct in 93% of cases (Petley, 1998). Not all cast antlers were recovered in the field, so the proportion of stags for which both antlers were found in a given year was relatively low. Yearling and 2-year-old stags typically produce only a pair of single spikes, rather than the more elaborate forms with branches (tines). Because this makes individual recognition of antlers difficult, measurements on younger individuals were rare, and so only individuals aged 3 years or older were considered for this analysis.

We analysed measures of four different antler traits

- 1 Length of the brow tine (mm): the lowest and most prominent branch of the antler.
- 2 Total length of antler (mm).
- 3 Total dry weight (g).
- 4 Number of points, including all branches (or tines) and the top points.

Measurements on parts of antlers that had been broken in fights or chewed since casting were not considered (both breakage and chewing are easily recognizable). Sample sizes varied between different analyses, because the set of traits measured varied between years and data on variables such as breeding success or multilocus heterozygosity (see below) were not available for all individuals.

### Measures of fluctuating asymmetry

The magnitude of absolute fluctuating asymmetry (FA) was assessed as the unsigned difference between the measurements of the left and right sides,  $|L-R|$ . We also considered measures of relative FA, estimated by dividing absolute FA by the average of both sides,  $\frac{1}{2}(L+R)$ , and expressed as a percentage. Both of these measures follow nonstandard distributions for all traits, so all statistical tests in models with FA as the dependent variable are based on randomisation procedures (details below).

### Environmental conditions

We tested for associations between FA and environmental conditions by relating FA to the following biotic and abiotic variables, all of which are known to affect

measures of performance in either the Rum red deer population or other northern ungulate populations.

#### *Population density*

As with previous studies of this population, we used as our measure of population density the number of adult (aged 1 year or more) females resident in the population. Population density has been shown to affect juvenile survival (Clutton-Brock *et al.*, 1987b; Rose *et al.*, 1998; Albon *et al.*, 2000), female lifetime breeding success (Clutton-Brock *et al.*, 1987a; Kruuk *et al.*, 1999a; Kruuk *et al.*, 1999b), male antler size (Schmidt *et al.*, 2001; Kruuk *et al.*, 2002), and a variety of other condition-related traits.

#### *North Atlantic Oscillation*

A large-scale atmospheric phenomenon, the North Atlantic Oscillation (NAO) is a significant determinant of both interannual variation and decadal trends in temperatures and precipitation during winter in northern latitudes, and shows frequent influences on growth, development, fecundity and demographic trends in several northern ungulate species (e.g. Forchhammer *et al.*, 1998; Post & Stenseth, 1999). Measures of the NAO are available from <http://www.met.rdg.ac.uk/cag/NAO/index.html> (Hurrell, 1995).

#### *Summer rainfall*

Rainfall during June and July has a significant effect on antler growth (Schmidt *et al.*, 2001; Kruuk *et al.*, 2002), presumably through a prolonged availability of high quality forage, which also enhances body weight gain (Langvatn *et al.*, 1996).

### **Measures of individual quality or condition**

Associations between individual quality and fluctuating asymmetry were tested using phenotypic measures and data on breeding success.

#### *Early development*

An individual's early development can have substantial effects on performance throughout its lifespan: birth weight affects juvenile survival (Clutton-Brock *et al.*, 1987b), yearling antler length (Schmidt *et al.*, 2001) and even adult lifetime breeding success (Kruuk *et al.*, 1999b), whereas birth date affects juvenile survival (Clutton-Brock *et al.*, 1987b) and yearling antler length (Schmidt *et al.*, 2001). We therefore tested whether birth date or birth weight showed any significant associations with fluctuating asymmetry.

#### *Breeding success*

We tested for effects of fluctuating asymmetry on an individual's breeding success, which will reflect behavioural dominance, fighting and roaring ability and ability to defend a harem of females against rivals.

Paternity was assigned using behavioural information on which male a female was associated with at the time she conceived, with time of conception estimated by back-dating from the calf's date of birth in the following spring. Annual breeding success (ABS) was defined as total number of calves fathered in a given year. Lifetime breeding success (LBS) was defined as the sum, across an individual's entire lifetime, of all measures of annual breeding success (restricted to individuals not still alive, and that had not been shot in the cull in adjacent parts of the island). The correlation between estimates of lifetime breeding success calculated using these behavioural data and those calculated using the genetic data for those individuals for which it was available was 0.86, but extensive genetic sampling did not extend back to the early part of the study period; see Kruuk *et al.* (1999b) for further details. Breeding success in a given year was compared with measures of FA in that year, and total lifetime breeding success was compared with the average of all measures of FA for a given trait across an individual's lifetime.

### **Multilocus heterozygosity**

The relative level of inbreeding in an individual's ancestry was assessed by its average heterozygosity at multiple loci: inbreeding reduces heterozygosity. Animals were typed at up to nine highly variable microsatellite markers (see Coulson *et al.*, 1998; Marshall *et al.*, 1998). An animal was only included in any analysis if it was typed at six or more loci. Because different individuals were untyped at different loci, we used a standardised measure of multilocus heterozygosity, defined as the ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed (Slate *et al.*, 2000).

### **Statistical analyses**

#### *Measurement error*

Measurement error can lead to overestimation of levels of FA, and several methods have been developed to quantify the effects of ME on FA, based on replicate measures of the focal traits. Here, we used repeated measures on antlers cast in 1999 to quantify levels of measurement error. Repeated measures on the same antler were not available for antlers collected prior to 1999, so we rely on an assumption that the 1999 estimate of measurement error is representative of the entire sample. We quantified measurement error in absolute FA using Merilä & Björklund's method I (1995), estimated from the proportion of within-individual variation in FA relative to the total variation (see also David *et al.*, 1999).

#### *Correlations between traits*

Correlations between every pairwise combination of the four traits were calculated using all observations on all

individuals. Correlations between measures of absolute antler size were tested using Pearson correlations, and between measures of FA using Spearman rank correlations. (This was because the absolute size of each antler trait followed a normal distribution, so the distribution of the unsigned differences between left and right values was clearly non-normal.)

#### Generalised linear mixed models (GLMMs)

Associations between FA, environmental conditions and individual quality were tested using multivariate models, with FA as the dependent variable and the different environmental or individual variables described above as independent variables. To account for the repeated measures on the same individuals across different years, all analyses were performed using GLMMs with individual stag as a random factor, using Genstat Version 5.4.1. In a GLMM, the significance of fixed effects is usually assessed from the Wald statistics, which are assumed to follow a  $\chi^2$  distribution, on the respective d.f. However use of these distributions relies on an underlying assumption of normality (or other standard parametrical distribution) in the error structure, which will clearly have been violated in the analyses of absolute FA. We therefore assessed the significance of effects by bootstrapping the GLMMs 10 000 times, and report the 95% confidence interval for the effect of each trait.

#### Estimates of variance components and heritability

Heritability values for absolute FA were estimated using an 'animal model' (Lynch & Walsh, 1998) and pedigree information on the total population, a total of 2374 individuals. As calves remain with their mothers for at least 1 year, maternity was easily determined from field observations (no conflicts with genetic data were encountered). Paternity was assigned for 840 calves using a combination of genetic and behavioural data, with offspring of individual males being distributed over several matriline: see Kruuk *et al.* (2000) for further details of pedigree construction.

We estimated heritability using a multivariate restricted maximum likelihood (REML) procedure in the

software package VCE (version 4, available from <http://www.tzv.fal.de/institut/genetik/vce4/vce4.html>). We fitted an animal model (a form of mixed model; Lynch & Walsh, 1998) in which the phenotype of each individual was broken down into a fixed effect for age, a random effect of additive genetic value and a random effect of permanent environment. This last effect accounts for permanent differences between individuals over and above those because of additive genetic effects; following convention, we refer to it as a permanent environment effect, although it will also incorporate any nonadditive genetic variance (strong epistatic genetics effects on FA have recently been reported for mandible size in mice: Leamy *et al.*, 2002). Deviations from normality in the distribution of absolute FA may affect optimality properties, but the estimates remain unbiased (Meyer, 1985; Höschele *et al.*, 1987). The total phenotypic variance was therefore partitioned into the following components:

$$V_{\text{TOT}} = V_{\text{A}} + V_{\text{PE}} + V_{\text{RES}}$$

where  $V_{\text{TOT}}$  is the total phenotypic variance,  $V_{\text{A}}$  is the additive genetic variance,  $V_{\text{PE}}$  is the permanent environment variance and  $V_{\text{RES}}$  is the residual variance. The narrow-sense heritability, or the resemblance between parent and offspring values, was estimated from the ratio of the additive genetic variance to the total phenotype variance:  $h^2 = V_{\text{A}} / V_{\text{TOT}}$ ; the permanent environment effect was estimated as  $\text{PE} = V_{\text{PE}} / V_{\text{TOT}}$ . VCE4 provides standard errors on these proportions.

## Results

### Description of fluctuating asymmetry and measurement error

Table 1 gives details of the distributions of each trait and its fluctuating asymmetry, the percentage measurement error in FA (Merilä & Björklund, 1995) and a  $P$ -value for the null hypothesis that the observed FA is simply measurement error (David *et al.*, 1999). For all traits, individual variation in FA was significantly greater than any within-individual variation generated by measurement error, and the average amount of absolute FA

**Table 1** Details of antler traits and fluctuating asymmetry. N obs. is the total number of FA measurements; N stags is the number of stags for whom measurements were available. Relative FA is expressed as a percentage of the average trait size. % ME in FA gives the percentage of variation in absolute FA because of within-individual variation and hence as a result of measurement error (based on repeated measures of 1999 antlers, using method I of Merilä & Björklund, 1995 – see Methods).  $P$ -value for individual differences is the significance of the between-individual variation in FA, i.e. not because of measurement error (based on repeated measures).

Trait	N obs.	N stags	Mean size (SD)	Mean Abs. FA (SD)	Mean Rel. FA (SD)	% ME in FA	$P$ -value indiv. diff.s
Brow (cm)	320	180	20.532 (4.753)	1.776 (1.698)	9.721 (11.423)	0.80	<0.001
Length (cm)	285	167	66.486 (10.902)	2.407 (2.535)	3.639 (3.767)	4.50	<0.001
Weight (g)	203	116	642.761 (231.239)	41.588 (36.469)	6.558 (5.180)	5.25	<0.001
Form (No. of points)	254	145	4.791 (0.965)	0.409 (0.531)	8.378 (10.956)	0	n.a.*

\*All variation as a result of between-individual differences.

**Table 2** Correlations between size and FA across traits. Correlations between trait average size above diagonals, and between absolute FA/relative FA below diagonal.

	Brow	Length	Weight	Form
Brow		0.555***	0.723***	0.498***
Length	-0.020 / 0.045		0.846***	0.373***
Weight	0.061 / 0.023	0.118 / 0.108		0.631***
Form	-0.043 / -0.060	0.027 / 0.017	-0.011 / -0.049	

\*\*\* indicates  $P < 0.001$ . None of the correlations below the diagonal were significant.

accounted for by measurement error ranged from 0 to 5.25% (method I, Merilä & Björklund, 1995), indicating that measurement error was small and therefore should not affect the analysis. We therefore tested the six hypotheses outlined in the introduction.

### Correlations between FA in different traits

Average size for each antler trait was strongly correlated with all of the other three measurements, with the highest correlation (0.846) being between dry weight and total length (Table 2). All six of the pairwise

correlations were significant at the 5% level, even after a sequential Bonferroni correction for multiple testing (Rice, 1989). In contrast, there were no significant positive correlations between levels of either absolute or relative FA for the four traits (Table 2).

### Fluctuating asymmetry and antler size

In contrast to the predictions outlined in the introduction, absolute FA increased with average size for three of the four traits: larger antlers had larger absolute asymmetry (Table 3a). There were no significant associations between relative FA and average size (Table 3b).

Antler size increases with age (Kruuk *et al.*, 2002), and the association between absolute FA and average size appeared to be driven by this age-related variation: absolute FA increased with age for three of the four traits (Table 4a). Again, however, there were no significant associations between relative FA and age (Table 4b).

We therefore tested whether there was evidence of reduced asymmetry in individuals with large antlers within the non-age-related variation, by fitting average size after age. For absolute FA, the results were inconsistent: after correcting for age, average size had a

**Table 3** Effect of antler size on FA. Results from GLMMs of (a) absolute FA and (b) relative FA against individual's average of left and right measures of each antler trait. Effect and SE are parameter estimates from the GLMMs. 95% confidence intervals are from bootstrapping.

Trait	(a) Absolute FA			(b) Relative FA		
	Effect	SE	(Lower 95% CI, Upper 95% CI)	Effect	SE	(Lower 95% CI, Upper 95% CI)
Brow	-0.0298	0.0200	(-0.0686, 0.0385)	-0.880	1.245	(-1.241, -0.394)
Length	0.0302	0.0139	(0.0026, 0.0542)*	-0.0106	0.0209	(-0.0499, 0.0329)
Weight	0.0572	0.0103	(0.0421, 0.0893)*	-0.00113	0.00157	(-0.00344, 0.00352)
Form	0.1264	0.0345	(0.0478, 0.1963)*	0.7812	0.7287	(-0.836, 2.212)

\* indicates an interval that does not include zero. See Methods for further details of model fitting. Sample sizes as in Table 1.

**Table 4** Effect of stag age and antler size on FA. Results from GLMMs of (a) absolute FA and (b) relative FA against individual's age in years; and of (c) absolute FA and (d) relative FA against average trait size, from a model that also included individual's age in years. Effect and s.e. are parameter estimates from the GLMMs. 95% confidence intervals are from bootstrapping.

Trait	(a) Effect of age on absolute FA			(b) Effect of age on relative FA		
	Effect	SE	(Lower 95% CI, Upper 95% CI)	Effect	SE	(Lower 95% CI, Upper 95% CI)
Brow	0.0945	0.0325	(0.0224, 0.176)*	0.0308	0.219	(-0.507, 0.666)
Length	0.141	0.0557	(0.0331, 0.274)*	0.0556	0.0837	(-0.091, 0.282)
Weight	4.667	0.983	(2.80, 7.29)*	0.0233	0.148	(-0.278, 0.387)
Form	0.0143	0.0121	(-0.0110, 0.0460)	0.0596	0.250	(-0.507, 0.703)
	(c) Effect of size on absolute FA, after correcting for age			(d) Effect of size on relative FA, after correcting for age		
Brow	-0.0746	0.0223	(-0.1275, -0.0057)*	-1.152	0.139	(-1.626, -0.644)*
Length	0.0112	0.0197	(-0.0444, 0.0505)	-0.0404	0.0296	(-0.121, 0.029)
Weight	0.0444	0.0151	(0.0176, 0.0885)*	-0.00275	0.00229	(-0.00595, 0.0032)
Form	0.128	0.0369	(0.0392, 0.195)*	0.8217	0.7786	(-0.946, 2.137)

\* indicates an interval that does not include zero. See Methods for further details of model fitting. Age was measured in years (range 3–15). Sample sizes as in Table 1.

**Table 5** Effect of environmental conditions during year of antler growth on FA. Results from GLMMs of (a) absolute FA and (b) relative FA against North Atlantic Oscillation (NAO), population density (Popn. dens.) and summer rainfall (Sum. rn.). Effect and SE are parameter estimates from the GLMMs. 95% confidence intervals are from bootstrapping; \* indicates an interval that does not include zero. See Methods for further details of model fitting. The models of absolute FA also included individual's age. Sample sizes as in Table 1.

Trait	Env. factor	(a) Effect of environmental conditions on absolute FA			(b) Effect of environmental conditions on relative FA		
		Effect	SE	(Lower 95% CI, Upper 95% CI)	Effect	SE	(Lower 95% CI, Upper 95% CI)
Brow	NAO	0.00377	0.0453	(-0.0892, 0.0992)	0.209	0.303	(-0.506, 0.663)
	Popn. dens.	0.00304	0.004175	(-0.00601, 0.00746)	0.0472	0.0261	(-0.0198, 0.0908)
	Sum. rn.	-0.000373	0.00114	(-0.00231, 0.00264)	-0.00789	0.00762	(-0.0242, 0.0137)
Length	NAO	-0.0844	0.0743	(-0.228, 0.0581)	-0.122	0.112	(-0.319, 0.097)
	Popn. dens.	-0.00113	0.00777	(-0.0130, 0.0183)	0.00304	0.0111	(-0.0125, 0.0368)
	Sum. rn.	0.00112	0.00203	(-0.0041, 0.004)	0.00191	0.00305	(-0.00646, 0.00691)
Weight	NAO	-0.795	1.251	(-3.640, 2.570)	-0.116	0.186	(-0.538, 0.364)
	Popn. dens.	0.1408	0.125	(-0.151, 0.342)	0.0276	0.0182	(-0.012, 0.0609)
	Sum. rn.	0.00670	0.0319	(-0.0697, 0.0967)	-0.00503	0.00474	(-0.0157, 0.00809)
Form	NAO	0.0229	0.0166	(-0.0231, 0.0585)	0.370	0.346	(-0.674, 0.974)
	Popn. dens.	0.00120	0.00256	(-0.00536, 0.00495)	0.0248	0.0517	(-0.122, 0.099)
	Sum. rn.	0.000341	0.000441	(-0.000582, 0.000841)	0.00486	0.00918	(-0.0145, 0.0162)

**Table 6** Effects of early development on FA. Results from GLMMs of absolute FA and relative FA against (a) (b) birth weight (kg) and (c) (d) birth date (days after 1 May). Effect and SE are parameter estimates from the GLMMs. 95% confidence intervals are from bootstrapping; \* indicates an interval that does not include zero. See Methods for further details of model fitting. Models of absolute FA also included individual's age. N obs. is total number of measurements of FA; N stags is number of individuals in the analysis.

Trait	N obs. / stags	(a) Effect of birth weight on absolute FA			(b) Effect of birth weight on relative FA		
		Effect	SE	(Lower 95% CI, Upper 95% CI)	Effect	SE	(Lower 95% CI, Upper 95% CI)
Brow	192 / 99	-0.04498	0.1187	(-0.198, 0.098)	0.101	0.8541	(-0.963, 1.114)
Length	175 / 98	0.08902	0.1179	(-0.183, 0.237)	0.0888	0.1694	(-0.361, 0.319)
Weight	133 / 73	0.1513	2.107	(-5.10, 4.15)	-0.0783	0.3224	(-0.963, 0.581)
Form	173 / 93	0.0523	0.0315	(-0.0155, 0.0999)	1.015	0.553	(0.141, 2.014)*
		(c) Effect of birth date on absolute FA			(d) Effect of birth date on relative FA		
Brow	258 / 135	0.00122	0.01073	(-0.0213, 0.0153)	0.0221	0.0736	(-0.079, 0.132)
Length	232 / 130	-0.00269	0.0159	(-0.0184, 0.0180)	-0.00199	0.0244	(-0.0256, 0.0301)
Weight	178 / 98	-0.1259	0.257	(-0.503, 0.349)	0.00155	0.0398	(-0.0624, 0.082)
Form	225 / 122	0.000340	0.00310	(-0.00323, 0.00720)	0.00336	0.0641	(-0.0715, 0.145)

\* indicates a 95% confidence interval that does not include 0.

negative effect for one trait (brow length), had no effect for another, and had a positive effect for the final two traits (Table 4c). Thus for only one trait was there any indication that, at a given age, larger antlers were more symmetrical. Relative FA in brow length also decreased in individuals with longer brow tines after correcting for the age effects, but there was no such pattern in any other trait (Table 4d).

Because of this increase in absolute FA with age rather than with average size, we included age in all subsequent models of absolute FA before fitting other variables. This was preferable to including average size because the latter would have been confounded with some of the environment-dependent variation for which associations with FA were being tested. Neither age nor

average size were included in the models of relative FA as their effects had already been incorporated in the standardisation.

### Environmental conditions and early development

There were no significant associations between absolute FA and either population density, the NAO or summer rainfall in the year of antler growth, for any of the four traits (Table 5a). Similarly, relative FA did not appear to vary with any of the environmental measures considered (Table 5b).

Birth weight did not show any significant association with absolute FA (Table 6a). However the increase in relative FA in form with birth weight was significant at

**Table 7** Effect of multilocus heterozygosity on FA. Results from GLMMs of (a) absolute FA and (b) relative FA against standardised multilocus heterozygosity. Effect and SE are parameter estimates from the GLMMs. 95% confidence intervals are from bootstrapping; \* indicates an interval that does not include zero. See Methods for further details of model fitting. Models of absolute FA also included individual's age. N obs. is total number of measurements of FA; N stags is number of individuals in the analysis.

Trait	N obs. / stags	(a) Effect of heterozygosity on absolute FA			(b) Effect of heterozygosity on relative FA		
		Effect	SE	(Lower 95% CI, Upper 95% CI)	Effect	SE	(Lower 95% CI, Upper 95% CI)
Brow	240 / 124	0.797	0.787	(-0.437, 2.10)	8.05	5.582	(1.00, 15.66)*
Length	221 / 120	0.852	1.135	(-1.21, 2.97)	1.448	1.698	(-1.56, 4.49)
Weight	162 / 87	14.82	17.05	(-16.7, 42.0)	4.51	1.99	(0.43, 8.56)*
Form	207 / 113	0.283	0.248	(-0.122, 0.713)	6.53	4.866	(-1.46, 15.36)

\* indicates a 95% confidence interval that does not include 0.

**Table 8** Effect of FA on (a) (b) annual breeding success, ABS and (c) (d) lifetime breeding success, LBS. (a) (b) ABS was fitted as the dependent variable in generalised linear mixed models (GLMMs) with negative binomial errors, with age and (a) absolute FA or (b) relative FA as independent variables; significance was assessed from the Wald statistic for the term, on 1 d.f. (c) (d) LBS was fitted as the dependent variable in generalised linear models (GLMs, because only one measurement per individual) with negative binomial errors and average lifetime (a) absolute FA or (b) relative FA as independent variables; significance was assessed from the deviance accounted for by the term ( $\Delta$  deviance), on 1 d.f.

Trait	N obs. / stags	(a) Effect of absolute FA on ABS				(b) Effect of relative FA on ABS			
		Effect	SE	Wald statistic	P-value	Effect	SE	Wald statistic	P-value
Brow	240 / 124	0.0236	0.06255	0.14	0.706	-0.00966	0.0102	0.90	0.343
Length	221 / 120	-0.0944	0.0501	3.55	0.059	-0.07491	0.0351	4.55	0.033*
Weight	162 / 87	0.00221	0.00297	0.56	0.456	-0.021	0.0218	0.93	0.336
Form	207 / 113	0.113	0.202	0.31	0.575	0.00301	0.00997	0.09	0.763
Trait	N stags	(c) Effect of absolute FA on LBS				(d) Effect of relative FA on LBS			
		Effect	SE	$\Delta$ deviance	P-value	Effect	SE	$\Delta$ deviance	P-value
Brow	109	0.059	0.204	0.098	0.754	0.0046	0.0182	0.089	0.765
Length	101	-0.359	0.155	5.998	0.014*	-0.0949	0.0399	5.835	0.016*
Weight	78	-0.055	0.220	0.084	0.772	-0.0483	0.0405	1.802	0.179
Form	88	-0.015	0.190	0.006	0.938	-0.0031	0.0178	0.028	0.867

\* indicates  $P < 0.05$ . N obs. is total number of measurements of FA; N stags is number of individuals in the analysis. (N stags for LBS is less because individuals that were still alive or had been shot were excluded).

the 5% level (Table 6b). There were no significant effects of birth date on either absolute or relative FA (Tables 6c, d).

### Inbreeding depression and associations with heterozygosity

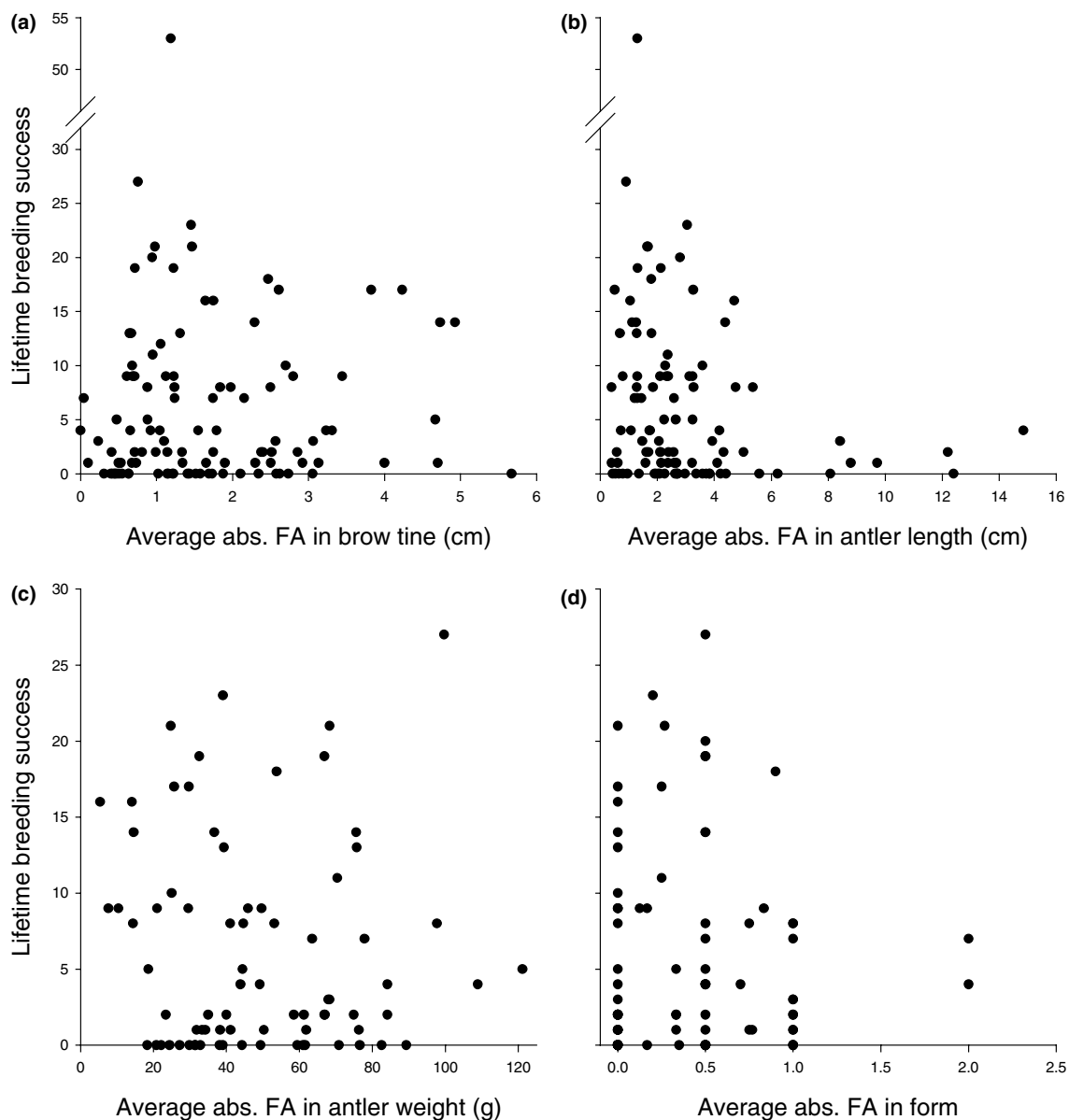
Standardised multilocus heterozygosity was not associated with levels of absolute FA in any trait (Table 7a), but there was a significant increase in relative FA in brow length and total weight with increased heterozygosity (Table 7b).

### FA as a predictor of breeding success

Annual breeding success was considered as the response variable in a GLMM with age (because breeding success varies with age, Kruuk *et al.*, 2002) and either absolute or relative FA as the independent variables. There were no effects for brow length, antler

weight or form (Tables 8a,b). However for total antler length, breeding success decreased marginally with increased levels of absolute FA ( $P = 0.059$ ), and decreased significantly with increased levels of relative FA ( $P = 0.033$ ; Table 8).

Secondly, we considered whether an individual's average FA across all years predicted his lifetime breeding success (averaging over age-corrected values for absolute FA, but not for relative). Again, breeding success decreased with increased asymmetry in total antler length (Tables 8c,d; Fig. 1). The lifetime breeding success of individuals with above-median absolute FA was reduced to 51% of those with below-median absolute FA (mean  $\pm$  SE LBS:  $8.23 \pm 1.41$  vs.  $4.25 \pm 0.72$ ). The effect of absolute FA in antler length was still significant after the exclusion of either the outlier in Fig. 1(b) – a highly successful stag named Maxi – or of those individuals for which measures of FA were only available for 1 year ( $\chi^2_1 = 4.674$ ,  $P = 0.031$ ;  $\chi^2_1 = 4.811$ ,  $P = 0.028$ , respectively).



**Fig. 1** Lifetime breeding success of individual stags and absolute FA in the four antler traits, averaged across all measurements on an individual. Brow length, total length and weight are corrected for age differences. Note differences in Y-axis scale between graphs because of differing availability of measurements.

### Heritability and permanent environment effects

Estimates of the components of variance and the heritability of absolute FA in each of the four traits are given in Table 9, and were not significantly different from zero in any case. Averaged across all traits, absolute FA had a mean heritability of 0.041, considerably smaller than the significant heritability observed in antler size (e.g. heritability of antler weight =  $0.350 \pm 0.109$ , Kruuk *et al.*, 2002). There was also no evidence of any significant permanent environment effect, reflecting the null results

of the tests in the above sections. Because of the lack of additive genetic variation, genetic correlations between traits were not calculated.

### Discussion

We have tested for a range of associations between measures of fluctuating asymmetry and environmental and phenotypic variables. In general, there was almost no evidence to support the hypotheses outlined in the introduction concerning FA in a secondary sexual trait.

**Table 9** Variance components and heritability of absolute FA. Estimates are from REML-based animal model, with age at antler growth as a fixed effect, and an additive genetic effect and a permanent environment effect as random effects.  $V_A$  is the additive genetic variance;  $V_{PE}$  is the component of variance because of permanent environmental differences between individuals;  $V_{RES}$  is the residual variance;  $V_{TOT}$  is the total variance;  $h^2$  is the heritability; PE is  $V_{PE}/V_{TOT}$ .

Trait	N obs.	N stags	$V_A$	$V_{PE}$	$V_{RES}$	$V_{TOT}$	$h^2$	SE	PE	SE
Brow	320	180	0.140	0.861	1.772	3.312	0.042	0.054	0.260	0.178
Length	285	167	0.443	0.726	5.476	8.903	0.050	0.108	0.082	0.134
Weight	203	116	0.006	13.203	1195.399	1319.837	<0.001	0.001	0.010	0.013
Form	254	145	0.006	0.016	0.109	0.131	0.044	0.080	0.121	0.093

Of the tests reported in the Tables 3–8, only four results lay in the predicted direction: a significant negative association between absolute FA in brow length and average brow length, after correcting for age effects; and three significant negative associations between the measures of FA in antler length and of annual and lifetime breeding success. Six further results were significant in the direction opposite to that predicted: three positive associations between absolute FA and antler size, two of which persisted even after age was included in the model; a positive association between relative FA in form and birth weight; and positive associations between relative FA and multilocus heterozygosity in two traits.

It seems intuitively reasonable that absolute FA might increase with the size of a trait, and hence that the increases with average size are realistic. However the three significant results amongst the tests for effects of environmental conditions or measures of individual quality on FA (Tables 5–7) make less sense. Given that three of 48 (6%) were significant at the 5% level, it seems likely that these may be because of Type I statistical errors: the inevitable rejection, in 5% of cases, of a correct null hypothesis. This suggestion is further supported by the lack of consistency in which trait was significant in any given analysis.

The one result with any consistency was the effect of FA in antler length on breeding success, which was significant or nearly significant ( $P = 0.059$ ) in all tests (Table 8). Fights between stags involve interlocking of antlers, so there may be mechanical reasons why asymmetry in antler length reduces fighting success. It is not possible to test directly whether the association between asymmetry in antler length and breeding success is generated by mutual correlations with individual quality, or whether antlers of different length are simply a less effective weapon in fights. In the latter case, the magnitude of the asymmetry may be entirely random and no reflection on any other characteristics of the individual, as is suggested by our set of null results for factors determining FA. FA in antler length will therefore be important because of the function of antlers, rather than because it reflects individual condition. However the lack of any effect of asymmetry in antler weight is also somewhat surprising, given that

selection on antler weight is stronger than on antler length (LK, unpubl. data), suggesting that antler weight is as or more important than length in determining fighting success.

It is also possible that further associations with FA were present but subtle in this population, and that our null results simply reflect a lack of statistical power in the analyses. For example, there has been much discussion of the problem of distinguishing between fluctuating asymmetry and measurement error (e.g. Palmer & Strobeck, 1986; Merilä & Björklund, 1995; David *et al.*, 1999). Measurement error in a trait will inflate estimates of the magnitude of FA, and may therefore obscure underlying signals in FA. However, the measurements of fluctuating asymmetry presented here had low measurement error: excluding the number of points (form), for which there was no measurement error, the average measurement error was 3.5%. This is considerably lower than, for example, the values presented by Merilä & Björklund (1995) for measurements of greenfinch morphology, which averaged 44% (range 17–99%) – presumably because measurement error will be relatively smaller for larger structures. There was also statistically significant variation between individuals in repeated measures of FA on the antlers grown in a given year, again implying that the observed variance in FA was not entirely because of measurement error. Furthermore, except in the highly implausible scenario that there were consistent differences between samples in measurement accuracy and that these differences were correlated with, for example, environmental conditions, measurement error will not have introduced any bias into the above analyses.

A lack of statistical power could also have been generated by insufficient sample sizes. However our sample sizes were relatively large for studies of FA and sexual selection: for example,  $N > 100$  is rare in the studies reported in Palmer's review of the literature (Palmer, 1999). Furthermore, although the magnitude of the effects was never significant, there was also no consistency in their direction. Finally, it could even be argued that our tests are biased towards detecting significant effects as we have made no correction of simultaneously testing multiple traits. We therefore believe it is unlikely that a lack of statistical power could explain the null results.

An alternative explanation for the lack of associations between FA and environmental conditions is simply that the environmental conditions were not sufficiently stressful (Hoffmann & Parsons, 1991). Although it will always be possible to induce more severe stress under artificial conditions than that experienced by a natural population, we believe we can reject this conclusion with respect to density, which has increased almost three-fold in terms of number of adult females since the cessation of culling in the study area in 1973. Increased density has reduced juvenile survival (Clutton-Brock *et al.*, 1987b; Coulson *et al.*, 1997; Albon *et al.*, 2000), delayed conception dates (Clutton-Brock *et al.*, 1987b), reduced female fecundity and total lifetime breeding success (Clutton-Brock *et al.*, 1987a; Kruuk *et al.*, 1999a, 1999b), skewed birth sex ratios (Kruuk *et al.*, 1999a), and reduced antler size (Schmidt *et al.*, 2001; Kruuk *et al.*, 2002). Similar effects have been documented in a range of other ungulate populations (reviewed in Gaillard *et al.*, 1998), all presumably generated by reduced resource availability at higher densities. Thus although the climatic variables considered – summer rainfall and the NAO – may have had more subtle effects, if FA was affected by any environmental conditions it should be reflected in associations with density. It is also worth noting that laboratory experiments on *Drosophila* also report a lack of consistent effects of environmental stress on FA in different traits (Woods *et al.*, 1999).

Thus there are no obvious explanations for our suite of null results, other than the conclusion that fluctuating asymmetry in antler size is not related to either individual quality or environmental conditions. For FA to be useful as any sort of indicator, it should at least be consistent across different traits measured on the same individual, even if the correlations are only weak (Whitlock, 1996). We found no evidence to suggest as much, even when looking at measurements of the same structure within an individual. There were also no associations between FA and any of a series of variables relating to performance or condition. Similarly, despite significant heritability in the Rum red deer population of several other condition-related traits, such as female fecundity or maturation age and male antler size (Kruuk *et al.*, 2000, 2002), there was no evidence of additive genetic variation underlying FA. Interestingly, in extensive studies of the genetic basis of FA in mouse mandible size, Leamy *et al.* (1997, 2002) found no evidence of significant additive genetic variance for FA, but did find substantial epistatic genetic effects (Leamy *et al.*, 2002). Here, however, there was also no indication of significant permanent between-individual differences, which would have reflected nonadditive genetic variation as well as permanent environment effects. Finally, although previous studies have documented significant associations between levels of inbreeding – as assessed by measures of genetic variability – and both survival and fecundity (Slate *et al.*, 2000, in preparation), multilocus heterozyg-

osity did not predict FA. We believe that our analyses were sufficiently broad and deep enough to detect any consistent pattern of FA. At least in this population, fluctuating asymmetry in antler size does not appear to reflect either environmental stress or individual genetic quality, and so is presumably a poor indicator of developmental stability. The weight of these null results suggests that the observed relationship between FA in antler length and breeding success is purely mechanistic, rather than representative of a deeper underlying association with individual condition.

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