

Patterns of fluctuating asymmetry in beetle horns: no evidence for reliable signaling

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Negative relations between trait size and levels of fluctuating asymmetry in secondary sexual traits have been claimed to be indicative of honest signaling of male quality. Comparative studies of beetle horns have been used to illustrate the required negative relation between trait size and asymmetry. However, such studies may be confounded by measurement error or sampling bias due to population differences or differences within species in the phenotypic expression of hornedness. We examined the patterns of fluctuating asymmetry within two species of horned beetle. We found that, in agreement with theory, horns exhibit greater asymmetry than naturally selected traits. However, we found a strong positive relation between horn size and asymmetry in *Onthophagus taurus*, a species with male dimorphisms, and a flat relation in *Bubas bison*, a species with continuous variation in horn size. We suggest that these differences may reflect functional differences in horns. We conclude that patterns of asymmetry in horned beetles do not support the notion of honest signaling. **Key words:** beetle horns, *Bubas bison*, fluctuating asymmetry, honest signaling, *Onthophagus taurus*, sexual selection. [*Behav Ecol* 9:465–470 (1998)]

Morphological traits differ in the degree to which they are developmentally canalized during development, according to their functional importance (Møller, 1993a; Møller and Pomiankowski, 1993; Palmer and Strobeck, 1986). Traits that have high functional importance are typically highly canalized and developmentally stable and thus demonstrate low levels of fluctuating asymmetry (FA). Traits that have less functional importance are frequently less stable and have higher levels of FA. This has been highlighted by an apparent dichotomy between secondary sexual and nonsexual morphological traits. Various studies have demonstrated that the degree of FA in male sexual ornaments and/or weapons generally exceeds that in other nonsexual morphological traits such as legs or wings (Møller, 1992; Møller and Eriksson, 1994; Møller and Hoglund, 1991; but see Balmford et al., 1993). Similarly, sexual traits in males generally possess higher levels of FA than homologous, and presumably nonsexual, traits in females (Manning and Chamberlain, 1993; Møller and Hoglund, 1991).

Møller (1993a) argued that the relationship between trait size and FA may be a potential indicator of individual quality. Individuals of low phenotypic and/or genetic quality are proposed to invest relatively more in the production of small traits, with the cost of high investment manifest as high levels of FA. In contrast, individuals of superior quality are proposed to invest relatively less and to be able to produce larger yet more symmetrical traits. Møller (1990, 1992, 1993a,b) and Møller and Hoglund (1991) thus propose that negative relations between trait size and asymmetry serve as honest signals of male quality. Negative relations between FA and trait size have been demonstrated for some taxa (Manning and Chamberlain, 1993; Manning and Hartley, 1991; Møller, 1990, 1993b; Møller and Eriksson, 1994, 1995) but not others (Allen and Simmons, 1996; Evans et al., 1995; Mateos and Carranza, 1996; Møller et al., 1996; Tomkins and Simmons, 1995).

In his comparative study of the patterns of FA in the horns and elytra of eight species of horned beetles, Møller (1992)

demonstrated that the degree of FA in horns was considerably larger than in elytra. In addition, he found that, on average, the relation between absolute FA and trait size was negative for horns and flat or U-shaped for elytra. From this Møller (1992) concluded that the patterns of FA in the horns of beetles may honestly signal male competitive ability. There are several problems with this study and with many of the studies of FA cited above. Rarely do these studies adequately account for measurement error which has statistical properties identical to FA, and often, particularly with comparative studies, they may be confounded by sampling bias; since both the level of FA and the size of the trait are often environmentally determined, a negative relation between FA and trait size might be expected as a confounding result of heterogeneous sampling (Simmons et al., 1995; Swaddle et al., 1994). One particular problem with Møller's (1992) study of beetle horns is that, within species males are often dimorphic for the expression of horns (Eberhard and Gutierrez, 1991; Otte and Stayman, 1979); only males of high phenotypic quality produce horns (Emlen, 1994; Hunt and Simmons, 1997) so that pooling data on FA across morphs may represent a form of sampling error (Tomkins and Simmons, 1996). This may be particularly true because, in beetles, horned males can represent a cohort of superior phenotypic quality (e.g., Brown and Barton, 1986; Cook, 1990; Emlen, 1994; Hunt and Simmons, 1997; Goldsmith, 1987), so that large horns and symmetrical horns may represent dichotomous, rather than continuous, conditions. Beetle horns are subject to strong sexual selection; horned males outcompete their hornless conspecifics (Emlen, 1997; Siva-Jothy, 1987) and, among horned males, those with longer horns are superior competitors (Conner, 1989; Emlen, 1997). To test Møller's (1992) findings regarding the levels and patterns of FA in horned beetles, we examined the relations between FA and trait size in the sexual and nonsexual traits within populations of both *Onthophagus taurus* and *Bubas bison* (Coleoptera: Scarabaeidae).

METHODS

We collected *Onthophagus taurus* from Narikup (34°45' S, 117°41' E), approximately 370 km south of Perth, Western Australia, and *Bubas bison* from Kojonup (33°51' S, 117°01' E), approximately 260 km south of Perth. Animals were collected by overturning fresh dung pads and extracting beetles

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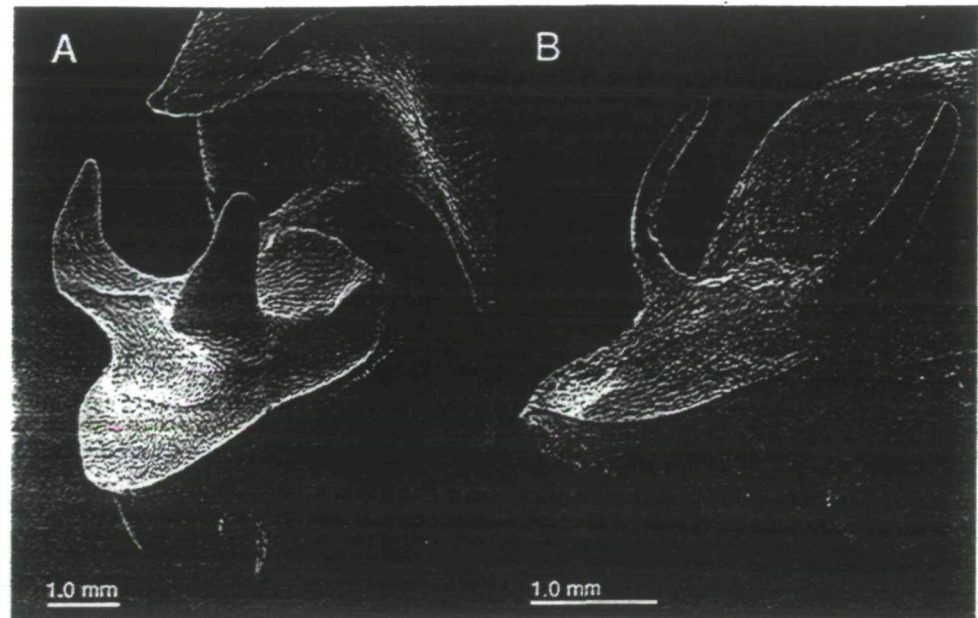


Figure 1
Scanning micrographs showing the horn structures of (A) *Bubas bison* and (B) *Onthophagus taurus*.

by hand as they attempted to bury into the underlying soil or move back through the pad. Beetles that escaped down their burrows were unearthed and also collected. For each species, animals were sampled from the same local population and on the same day.

Patterns of trait size and FA

For *O. taurus*, we measured size and FA in the paired head horns and the elytra of males and in the elytra of females. In *B. bison* FA was measured in the paired head horns and hind femur of both males and females. We also measured the size of the central pronotal horn of male *B. bison* (Figure 1). In each case FA was calculated by subtracting the left from the right side. The selection of morphological traits was based on the following criteria: (1) ease of measurement, which was largely determined by the presence of well-defined measurement landmarks, (2) traits that were less vulnerable to damage (e.g., hind femurs were less frequently worn or damaged than fore femurs), (3) both trait size and FA had to be measured with a high repeatability and a low level of measurement error, and (4) traits being used in FA examination had to conform to the statistical guidelines of FA (Swaddle et al., 1994). Where possible we measured the homologous traits in females to ascertain the differential selective pressures acting on the sexes. In both species, pronotum width was measured as an index of body size.

All FA measurements were made twice. Measurement error was estimated for the level of signed FA in bilateral traits following method 1 of Merilä and Björklund (1995). FA measures are especially sensitive to measurement error because the magnitude of FA is usually small (often $\leq 1\text{--}5\%$ of the total variation in a given trait) and is random by definition. Therefore, more than a single measurement is required to accurately assess the magnitude of FA relative to measurement error (Merilä and Björklund, 1995; Yezzerinac et al., 1992). Sexes, and where present male morphs (see below), were treated separately.

We assessed the presence of significant FA using procedures recommended by Palmer (1994). A mixed-model ANOVA using sides (left or right), individuals, and replicate entered as main factors provides an interaction variance, individual-by-sides, that is an estimate of the asymmetry variance. This was

compared with the variance due to measurement error (see also Swaddle et al., 1994). Traits were accepted as showing FA, rather than antisymmetry, if the signed asymmetries were normally distributed (determined by a Kolmogorov-Smirnov goodness-of-fit test) around a mean of zero (determined by a one-sample *t* test) or, if they did differ from normality, they only did so through leptokurtosis and not platykurtosis or skewness (Palmer and Strobeck, 1986). Leptokurtosis in the adult population is expected to arise through selection operating against asymmetric individuals during the course of development (Naugler and Leech, 1994; Ueno, 1994). In contrast, platykurtosis is likely to reflect a developmental bias generating antisymmetry (Palmer and Strobeck, 1986). If FA was non-normally distributed, both skewness and kurtosis were tested to determine the underlying cause. The statistical properties of our FA measures are given in Table 1.

We used mixed-model ANOVAs to control for measurement error variance when examining differences in FA between traits or between species. Variance ratio tests were performed using degrees of freedom calculated according to Palmer (1994). For heuristic purposes, we present the mean absolute values of FA or relative FA to illustrate the observed differences between samples, in addition to the measurement error-corrected variances in FA obtained from the mixed-model ANOVAs (measures FA_1 , FA_2 , and FA_{10} , respectively; see Palmer, 1994). Means are presented ± 1 SE.

RESULTS

Horn morphology

Variation in horn size differed between the two species examined. There was sigmoidal variation in horn height with increasing pronotum width in *O. taurus* (Figure 2) and continuous variation in horn height with increasing pronotum width in *B. bison* (Figure 3). These different relations suggest that male *O. taurus* are dimorphic for horn morphology; whereas *B. bison* exhibit continuous growth. The existence of male dimorphisms was tested statistically using the models outlined by Eberhard and Gutierrez (1991).

First, the relationship between pronotum width and horn height in male *O. taurus* and horn height and pronotal horn height in *B. bison* were examined to assess nonlinearity across

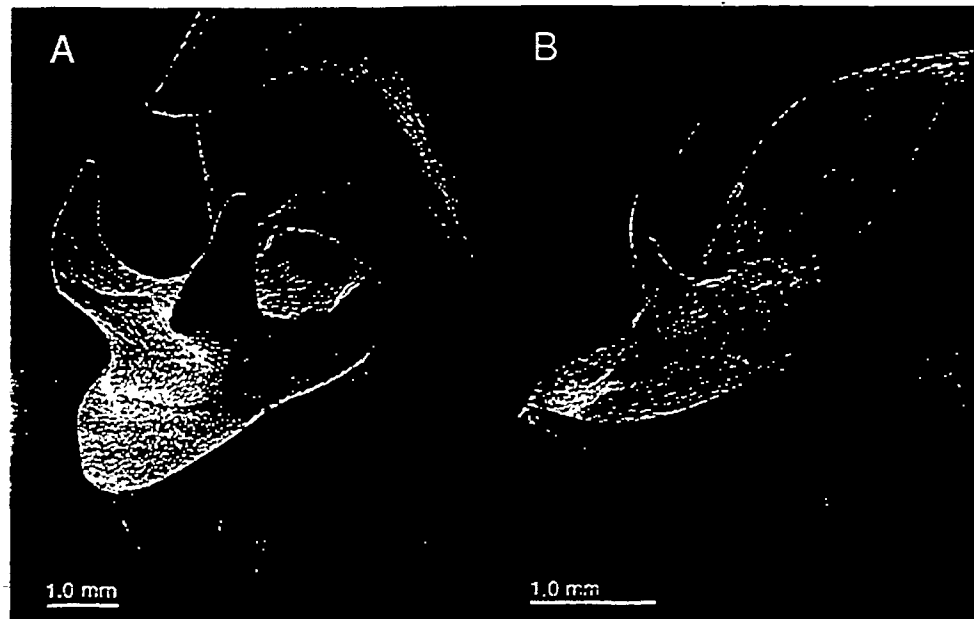


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Table 1
 Statistical properties and measurement errors (ME) of signed fluctuating asymmetry measures (mm) in *O. taurus* and *B. bison*

Species	F (df)	ME (%)	Skewness (g ₁)	Kurtosis (g ₂)	Mean (±SE)
<i>O. taurus</i>					
Major males					
Horn height	2.72 (52, 106)***	3.06	-0.100	0.502	0.002 ± 0.007
Elytra length	1.64 (52, 106)**	12.21	-0.032	1.676*	0.000 ± 0.001
Minor males					
Horn height	1.57 (82, 166)**	4.70	0.234	1.560**	-0.000 ± 0.002
Elytra length	1.78 (82, 166)***	8.24	0.007	0.879	-0.001 ± 0.001
Females					
Elytra length	1.69 (99, 200)***	20.06	0.017	1.807***	-0.000 ± 0.001
<i>B. bison</i>					
Males					
Horn height	12.86 (82, 166)***	3.75	-0.010	3.653***	-0.005 ± 0.006
Hind femur length	1.54 (82, 166)**	12.25	0.152	0.831	0.002 ± 0.002
Females					
Paired horn height	3.45 (89, 180)***	12.97	-0.401	0.573	0.000 ± 0.000
Hind femur length	1.98 (89, 180)***	10.27	0.179	0.996*	0.000 ± 0.000

F ratios derived from mixed-model ANOVAs contrasting fluctuating asymmetry variance with measurement error.

*** $p \leq .001$, ** $p \leq .01$, * $p \leq .05$.

males. Nonlinearity is expected if there is a critical body size after which beetles switch to producing a major body plan (Eberhard and Gutierrez, 1991; Emlen, 1994; Otte and Stayman, 1979). A partial regression of \log_e (pronotum width) and $[\log_e (\text{pronotum width})]^2$ on \log_e (horn height) generates two partial regression coefficients (ω_1 and ω_2), where an ω_2 that differs significantly from zero confirms nonlinearity and the possible existence of a switch point (Eberhard and Gutierrez, 1991; Emlen and Wrege, 1992). The data for both *O. taurus* (horn height: $\omega_2 = 32.89 \pm 14.82$, $t = 2.536$, $df = 147$, $p = .021$; Figure 2) and *B. bison* (horn height: $\omega_2 = -14.55 \pm 2.86$, $t = 5.082$, $df = 92$, $p < .001$; pronotal horn height: $\omega_2 = -12.38 \pm 2.55$, $t = 4.857$, $df = 92$, $p < .001$; Figure 3) showed significant deviations from linearity. Therefore, the data from both species was examined for the presence of a switch point, a pronotum width at which beetles switched from producing minor horns to major horns. This was accomplished by extending the partial regression equa-

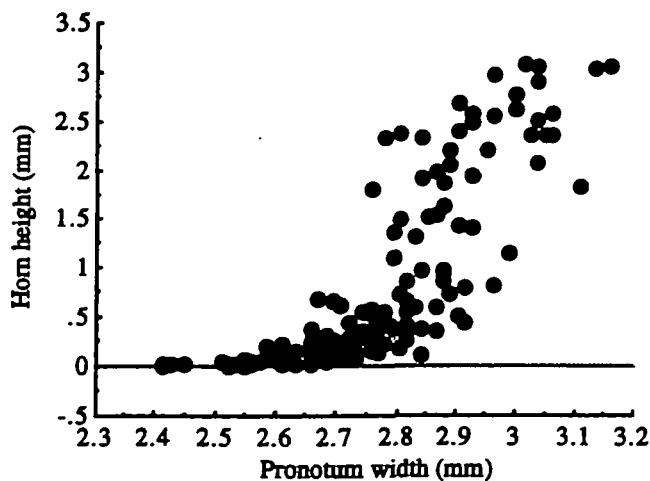


Figure 2
 Relation between male body size and horn size in *Onthophagus taurus*.

tion to $Y = \beta_0 + \beta_1 X + \beta_2 (X - X^*)D + \beta_3 D + e$, where Y = horn height, X = pronotum width, X^* is the proposed switch point where $D = 0$ if $X < X^*$, otherwise $D = 1$, β_i are the regression coefficients, and e is the random component with assumed normal distribution, mean zero, and a common variance (Eberhard and Gutierrez, 1991). To determine which switch point gave the best fit, 10 different hypothetical switch points were tested, ranging from 2.72 to 2.85 mm in *O. taurus* and from 5.4 to 6.2 mm in *B. bison*. These produced a range of adjusted r^2 values (*O. taurus*: .595-.642; *B. bison*: .38-.72) from which the pronotum width corresponding to the maximum r^2 value was taken as the most likely switch point. The highest adjusted r^2 occurred at a pronotum width of 2.783 mm in *O. taurus*, and ranged between 5.863 and 5.923 mm in *B. bison*. In *B. bison* the middle value of the adjusted r^2 range (5.893 mm) was used in subsequent tests for dimorphism.

Using the best switch point values for both *O. taurus* and *B. bison*, we tested the data to determine if the variation in horn size was continuous or discontinuous at the hypothetical switch point (null hypothesis: $\beta_3 = 0$) (Eberhard and Gutierrez, 1991). β_3 was found to be significantly > 0 in *O. taurus* (horn height: $\beta_3 = 0.34 \pm 2.54$, $t = 2.536$, $df = 147$, $p = .012$), demonstrating a discontinuity between male morphs at the switch point. β_3 in both horn height and pronotal horn height of *B. bison* did not differ significantly from zero (horn height: 0.07 ± 0.06 , $t = 1.133$, $df = 92$, $p = .26$; pronotal horn height: -0.08 ± 0.09 , $t = 0.868$, $df = 92$, $p = .39$), which suggests that variation in these male traits were continuous rather than discontinuous.

Testing the change of linear slope of horn height versus pronotum width at the switch point confirms the existence of a significant switch point in *O. taurus* (null hypothesis: $\beta_2 = 0$; $\beta_2 = 5.24 \pm 0.90$, $t = 5.838$, $df = 147$, $p < .001$). As a result, *O. taurus* can be separated into two discrete male morphs, with males having a pronotum width ≥ 2.783 mm being classed as major morphs and males with a pronotum width < 2.783 mm being classed as minor morphs. All subsequent analyses are based on these classifications. In contrast, *B. bison* males did not show two discrete male morphs but instead demonstrated a nonlinear, continuous relation between horn and body size.

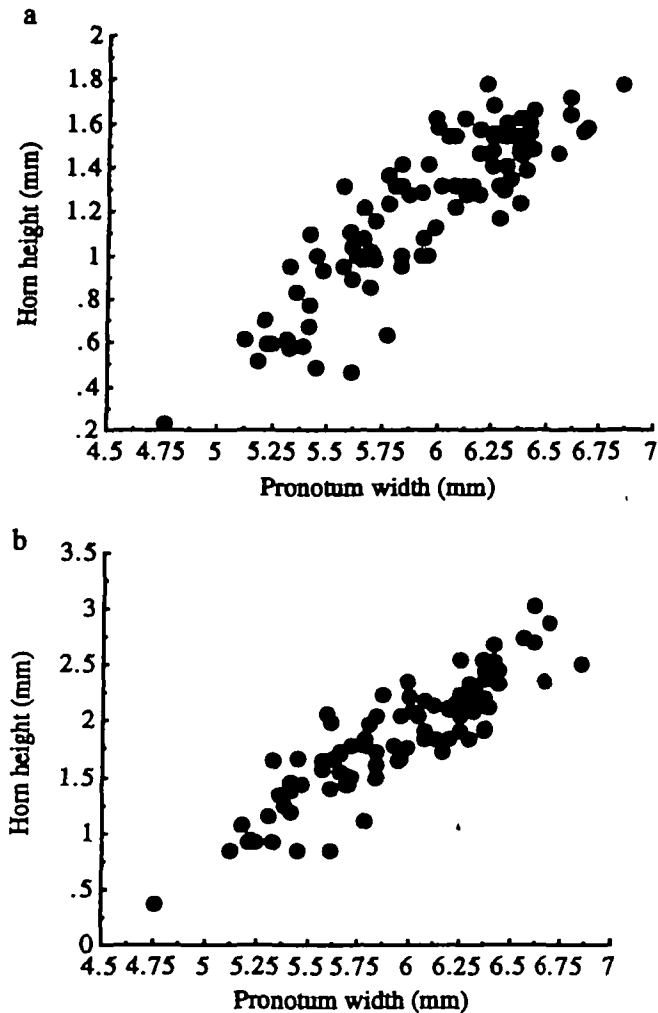


Figure 3
Relation between male body size and (a) paired head horn size and (b) pronotal horn size in *Bubas bison*.

Patterns of FA

Spearman rank correlations revealed a significant positive relation between absolute FA and horn size within both morphs of male *O. taurus* (Table 2). Similarly, a significant positive relation between FA and paired horn size was observed in female *B. bison* (Table 2). No significant relations between absolute FA and trait size were demonstrated in any of the other morphological traits examined (Table 2). The above analyses do not partition measurement error from FA variance. Mixed-model ANOVAs can be used to partition FA variance when comparing populations, but this method cannot provide measures of FA for among individual analyses (Møller and Swaddle, 1997). Nevertheless, given significant repeatability of the signed FA measures (Table 1) and the calculation of mean values of absolute FA from repeated measures of all individuals, our analyses should provide reasonable approximations to the true relationships (see below).

We can control for measurement error in comparisons between samples. After partitioning measurement error variance, horn FA was greater in major male *O. taurus* than in minor males ($F_{12, 8} = 8.54$, $p = .008$; Table 2). This is consistent with our among-individual analysis, where FA increased with horn size. To control for allometric scaling of FA with horn size, we divided each of the left and right horn measures by the overall mean horn size (i.e., the mean of our two left

and two right measures) and log transformed the product. The effectiveness of this scaling was confirmed by assessing the significance of the variance contribution due to individuals over the individual-by-side variance from mixed-model ANOVAs (Palmer and Strobeck, 1986). Although there was no relation between elytra length and FA in either morph (Table 2), for consistency we used the same scaling procedure throughout. Relative horn FA was greater in major males than in minor males ($F_{30, 28} = 37.17$, $p < .001$); the relative FA in elytra did not differ between morphs ($F_{10, 9} = 1.13$, $p = .484$).

We used the same scaling procedure for our between-trait comparisons. Relative FA was consistently greater in the horns of males than it was in their corresponding nonsexual traits (*O. taurus* majors $F_{30, 9} = 335.09$, $p < .001$; minors $F_{28, 10} = 8.00$, $p < .001$; male *B. bison*: $F_{72, 9} = 291.55$, $p < .001$). The horns of male *B. bison* had greater relative FA than the homologous trait in females ($F_{72, 10} = 532.99$, $p < .001$). Moreover, the horns of females were no more asymmetrical than their hind tibia ($F_{21, 10} = 1.56$, $p = .239$; Table 2). The critical Bonferroni probability for the five variance ratio calculations performed using data for *O. taurus* ($p_{.05} = .01$) and three variance ratio calculations performed using data for *B. bison* ($p_{.05} = .017$) were greater than the individually significant probabilities in all cases.

DISCUSSION

The data presented here for *O. taurus* and *B. bison* are in agreement with the notion that sexually selected characters demonstrate higher levels of FA than do nonsexual traits (Manning and Chamberlain, 1993; Møller, 1992; Møller and Eriksson, 1994; Møller and Hoglund, 1991; but see Tomkins and Simmons, 1995). Møller (1992) found similar patterns in his comparative study of beetle horns, where the levels of relative FA in horns were nearly six times as large as in nonsexual elytra. Møller's (1992) analysis did not distinguish between measurement error and FA. Björklund and Merilä (1997) suggest that the estimates of FA in Møller and Hoglund's (1991) study of avian feather ornaments may be inflated by an average of 526%, so the differences observed between sexual and nonsexual traits may simply reflect the fact that sexual traits are harder to measure. However, after removing variance due to measurement error from our analysis, we still found that the levels of FA in beetle horns were considerably higher than in elytra. Disparate levels of FA in morphological traits have been proposed to reflect the different nature of selection operating upon them (Møller, 1993a). Natural selection against FA in mechanically functional traits, such as elytra or legs, is expected to favor the action of genetic modifiers that canalize developmental processes and reduce phenotypic variance (Soulé, 1982; Soulé and Couzin-Roudy, 1982). Conversely, because secondary sexual traits are typically subject to strong directional selection, which disproportionately favors individuals with greater than average values, selection is expected to oppose genetic modifiers that canalize development, thereby increasing phenotypic variance and hence levels of FA (Møller, 1993a).

Møller (1992) concluded that beetle horns were an honest signal of male competitive ability because those individuals with large horns were also able to produce more symmetrical horns. In general, a negative relation between trait size and symmetry has been predicted for traits that honestly signal individual quality (Møller, 1992; Møller and Hoglund, 1991). However, our data provide no evidence for the honest signaling hypothesis within the species of horned beetles that we studied. In contrast to Møller's (1992) comparative study, we found that for *O. taurus* there was a strong positive relation between horn size and FA for both major and minor morphs,

Table 2

Mean absolute and relative fluctuating asymmetry (FA) and their variances after partitioning measurement error for the bilaterally paired traits of *O. taurus* and *B. bison*

Trait	Absolute FA		Relative FA		r_s	n
	Mean	σ^2	Mean	σ^2		
<i>O. taurus</i>						
Minor						
Horn height	0.010	6.54×10^{-5}	0.040	3.99×10^{-6}	0.664**	83
Elytra length	0.006	2.55×10^{-5}	0.002	4.99×10^{-7}	-0.041	83
Major						
Horn height	0.045	5.58×10^{-4}	0.035	1.48×10^{-4}	0.711**	53
Elytra length	0.005	1.92×10^{-5}	0.001	4.43×10^{-7}	-0.222	53
Female						
Elytra length	0.005	1.82×10^{-5}	0.002	3.84×10^{-7}	-0.074	100
<i>B. bison</i>						
Male						
Horn height	0.032	1.56×10^{-3}	0.027	1.89×10^{-4}	0.081	83
Hind femur length	0.014	1.73×10^{-4}	0.003	6.49×10^{-7}	-0.160	83
Female						
Paired horn height	0.010	8.29×10^{-5}	0.099	3.55×10^{-7}	0.240*	90
Hind femur length	0.011	7.52×10^{-5}	0.002	5.52×10^{-7}	-0.133	90

Spearman rank correlation coefficients (r_s , values corrected for ties) show the relations between trait size and absolute asymmetry.

** $p < .001$, * $p < .05$.

whereas for *B. bison* the relationship was flat. The paired head horns of *O. taurus* are used either to block the entrance to breeding tunnels or to push against intruding males. Males with longer horns have a competitive advantage over males with smaller horns (Moczek, 1996; see also Emlen, 1997). In the absence of selection against FA, selection for increased trait size is expected to generate positive size-dependent asymmetry (Møller, 1993a). In contrast, the paired head horns of *B. bison* articulate with the single pronotal horn in a manner similar to that noted in *Podischnus agenor*, where the horns function as a grasping apparatus used in conventional disputes between males (Eberhard, 1979) (Figure 1). Natural selection should oppose FA in such structures to ensure their mechanical function can be performed effectively. We believe that, in general, the functionality of secondary sexual traits may prove important in explaining differences in the observed influence and patterns of FA. Flat relations between trait size and asymmetry have been reported for other secondary sexual traits that serve a mechanical function, such as the forceps of earwigs (Tomkins and Simmons, 1995) and the wing clamps of sepsid dungflies (Allen and Simmons, 1996).

Our results raise an important issue concerning the observed negative FA-trait size relationships reported in other studies. We found that measurement error for signed FA was greater for small horns than for large horns; in *O. taurus* measurement error for minor male horns was 1.5 times that for major male horns, whereas the small horns of female *B. bison* had 4 times the measurement error of male horns. Negative size-biased measurement error appears to be quite common in the measurement of morphological characters (Yezerinac et al., 1992). The fact that absolute FA always has higher measurement error than signed FA (Björklund and Merilä, 1997; Merilä and Björklund, 1995) compounds the problem. Thus, measures of absolute FA for small traits will be inflated by measurement error to a greater degree than those of large traits, so that a negative relationship between absolute FA and trait size may reflect size-dependent measurement error. Our observation of positive relationships between absolute FA and

horn size in *O. taurus* are thus likely to be conservative because our estimates of absolute FA in small horns will be inflated by measurement error to a greater extent than our estimates of absolute FA in large horns. To our knowledge, no other studies have accurately assessed the effects of measurement error when examining patterns of FA with trait size.

In conclusion, although the patterns of FA observed in the sexual and nonsexual traits of *O. taurus* and *B. bison* are in general agreement with the prediction that naturally selected traits are typically more highly canalized during development, the patterns of FA in beetle horns are inconsistent with the honest signaling hypothesis (Møller, 1992, 1993a; Møller and Pomiankowski, 1993). Recent studies show that negative relations between trait size and FA can characterize bilaterally paired traits when asymmetry is not subject to sexual selection (Polak, 1997; Veiga et al., 1997). Conversely, traits that are both indicative of male quality and subject to sexual selection may not show the predicted negative relations (Kimball et al., 1997; Markow et al., 1996). Rowe et al. (1997) have shown that most studies supporting the notion that negative size-dependent asymmetry reflects individual quality in fact represent cases of antisymmetry rather than FA. Moreover, they note that, unlike antisymmetry, FA is unlikely to be a reliable signal because symmetrical individuals will be common in classes of individuals in poor condition. The resolution of conflicting evidence clearly lies in a better understanding of the development and nature of the observed asymmetries in secondary sexual traits and a more accurate partitioning of measurement error from FA data.

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