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Density dependence of developmental instability in a dimorphic ungulate

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The use of fluctuating asymmetry (FA) for biomonitoring environmental stress is limited by the lack of work on how FA in particular traits responds to specific stresses. Here, by manipulating the number of individuals in an enclosed fallow deer (*Dama dama*) population, we describe, for the first time, clear density dependence in the FA of juvenile jaw morphology. The impact of high population density on FA was strong for both sexes, supporting the use of FA for indexing environmental stress. In addition, there was some indication that the change in FA was greater in males (43.6%) than females (28.5%). Finally, the ability to buffer density-dependent stress was independent of body condition. We suggest that, under highly limiting conditions, whole cohorts may be unable to buffer against developmental error, irrespective of individual quality.

Keywords: body condition; fluctuating asymmetry; sexual dimorphism

1. INTRODUCTION

Change in non-directional departures from perfect symmetry (fluctuating asymmetry, FA) has often been suggested as an indicator of the environmental (Parsons 1990) or genetic (Leamy & Klingenberg 2005) stress experienced by individuals and populations. However, there is still no clear pattern in the FA–stress relationship from field studies, mainly (i) because there is often a lack of information on the exact intensity of stress and (ii) due to spatio-temporal variation in the stressor agents to which organisms are exposed (Bjorksten *et al.* 2000). For these reasons, work under controlled conditions is essential to develop a general framework that predicts when and what asymmetry–stress associations can be expected (Lens *et al.* 2001). Experimental work has much improved the state of the art on biomonitoring concerning FA–stress associations in invertebrates (Hoffmann & Woods 2003); however, we still lack equivalent information on the FA–stress association in vertebrates, and especially for large mammals.

High population density induces stress by increasing intraspecific competition for food (Stewart *et al.* 2005). As individual metabolic requirements are independent of animal density, increments in population density at constant food supply impose a handicap for organism homeostasis (Damuth 1981). In dimorphic species, because males and females show sex-specific trade-offs between growth and reproduction, the consequences of density dependence are expected to be sex specific (Stamps 1993). Commonly, males are heavier, require more energy for growth and mature at larger size than females; hence, phenotypic depression occurs at lower environmental pressures for males (Carranza *et al.* 2004). While density-dependent effects on phenotypic characters have been widely reported in many ungulates (e.g. Forchhammer *et al.* 2001), there is almost no information on the response of developmental stability to changes in population density. Further studies of FA in ungulates have generally focused on antlers (e.g. Ditchkoff *et al.* 2001), which are regrown annually and are absent in females of most species. Skeletal parts are more likely to reflect developmental stability during early growth, and jaw morphology is important for efficient processing of food (Gordon *et al.* 1996) and is probably highly conserved. Hence, small deviations from symmetry in jaw dimensions probably indicate developmental problems provoked by substantial environmental stress.

By manipulating the number of fallow deer (*Dama dama*) in an enclosed population, we generated years of contrasting density to evaluate density dependence in the ability of young animals to buffer against developmental instability of jaw bones. The fallow deer is a slow-growing, sexually dimorphic mammal (males 40% heavier than females; Feldhamer *et al.* 1988). Although birth weight is similar in the two sexes, young males grow faster (Birgersson & Ekvall 1997) and need more energy and protein than females (Ru *et al.* 2003). We examine three predictions: (i) intraspecific competition at very high density decreases developmental stability and so increases FA, (ii) because males are more sensitive to environmental stress, the effect of high density on FA is more pronounced among males than females, and (iii) individuals in better condition are able to buffer more effectively against density-dependent stress and hence express lower FA.

2. MATERIAL AND METHODS

(a) *Study area and population*

The study enclosure (130 ha) is situated in the northeast of Toulouse, France (43°46′ N, 1°35′ E). In 1980, seven fallow deer were released into the enclosure. From 1992 to 1994, animals were caught by darting (Zoletil) and marked with colour-coded collars to accurately determine population size using capture–mark–recapture methods. During this period, the population grew from 78 to 120 individuals, but then, due to changes in management requirements, the deer population was almost eradicated over a period of 2 years with no selective culling.

(b) *Study conditions*

At peak population size (92 deer km^{-2}: very high-density period), a first cull was carried out in winter 1993, which removed 82 animals (68% of the population). Over the following 2 years (average of 17 deer km^{-2}: moderate-density period), almost the whole of the population was removed by culling 30 deer (of 38 in the population) and then a further 8 deer in winters 1994 and 1995, respectively. Because the area was fenced, we assume that food resources were limited during the very high-density period compared with the moderate-density period. In addition, in the context of the expected low genetic variability of this closed population, we assume that the genetic influence on FA (Leamy & Klingenberg 2005) was similar in both periods.

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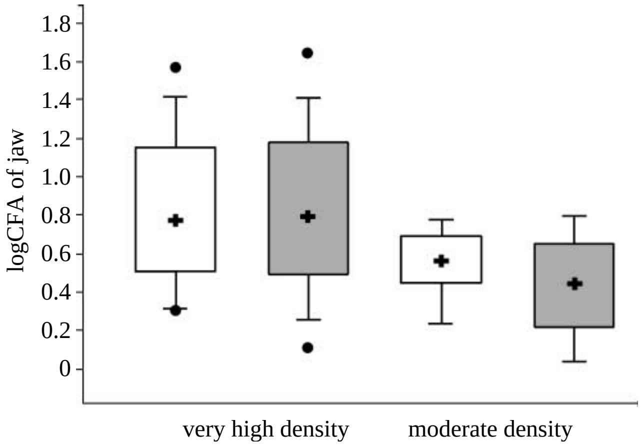

Figure 1. Density dependence of CFA (log-transformed) among juvenile female (white, $n=33$) and male (grey, $n=41$) fallow deer. The black plus indicates mean CFA; circles are outliers.

(c) *Fallow deer data*

To include only animals of known age, we considered fawns (more than 5 months old) and yearlings (1.5 years old), totalling 47 deer (17 fawn and 5 yearling females, and 16 fawn and 9 yearling males) in the very high-density period and 27 deer (7 fawn and 4 yearling females, and 7 fawn and 9 yearling males) in the moderate-density period. Jaws were removed and boiled in a 1% potassium hydroxide (KOH) solution. We measured two mandible traits in order to generate a composite index of unsigned FA (CFA2, see Leung *et al.* (2000) in the electronic supplementary material). In dimorphic cervids, changes in kidney fat reverses are good indicators of the individual's ability to cope with changes in resource limitation (Kaji *et al.* 1988). Thus, we used kidney fat reserves (following Serrano *et al.*'s 2008 recommendations) as a proxy for condition in fallow deer.

(d) *Statistical analysis*

We used CFA2 of the jaw as the dependent variable, sex and density as fixed factors (two modalities each) in a set of linear models (note, using density as a continuous variable and including a term for age class (fawns versus yearlings) did not alter our final conclusions, results not shown). We first tested the main effects of sex and density and their two-way interaction on CFA2. Second, to correct for the potential sex and density dependence in body condition, we took the residuals from the regression of the kidney fat on kidney weight, including the effects of sex and density on this relationship, and then tested the relationship between this body condition index and composite fluctuating asymmetry (CFA). We performed model selection based on the Akaike information criterion corrected for small sample sizes (Burnham & Anderson 2002) using R v. 2.5.1 (R Development Core Team 2007).

3. RESULTS

Following the reduction in density, our best model supported the hypothesis that FA was lower in both sexes (by 43.6% in males: very high density$=0.78\pm0.42$, moderate density$=0.44\pm0.25$; and by 28.5% in females: very high density$=0.77\pm0.39$, moderate density$=0.55\pm0.18$; figure 1, $F_{1,70}=4.68$, $p=0.034$). A sex difference in the fall of CFA in favour of males was suggested by the second best model that also received substantial support (interaction density×sex, ΔAICc$=0.44$; table 1, $F_{1,69}=1.33$, $p=0.253$). These two models together had a combined probability of 0.74 (sum of Akaike weights) to provide the best fit to the observed data. By contrast, we did not find substantial support for a relationship between CFA and individual body condition (AICc of model including reserves term$=227.15$ versus AICc of constant model$=228.11$, ΔAICc$=0.96$; see electronic supplementary material, figure 2).

Table 1. Model selection for FA of jaws in juvenile fallow deer ($n=74$). (K, number of parameters, including intercept and error term; AICc, Akaike information criterion corrected for small sample size; ΔAIC, difference of AICc with respect to the best model; *Wi*, relative probability that the model i is the best model given the observed data; Mo, constant model with the intercept and error term. In italics, models with substantial support.)

model	K	AICc	ΔAICc	*Wi*
density	3	72.59	0	0.41
density×sex	5	73.03	0.44	0.33
density+sex	4	75.01	2.42	0.12
Mo	2	75.62	3.03	0.09
sex	3	76.99	4.40	0.04

4. DISCUSSION

Our results provide clear evidence for density dependence in the CFA of jaws of a dimorphic ungulate. Thus, in line with our first prediction, developmental stability was sensitive to density-related environmental stress. In fact, the change in CFA between periods (reduction of developmental instability) was much greater than the change in absolute jaw size (the maximum recorded increase in size was 7.7% for the GD trait in yearling males; see the electronic supplementary material). Despite our moderate sample size, this is, to our knowledge, the first demonstration of density-dependent developmental stability in a non-sexually selected trait of a large mammal.

In contradiction of our second prediction, this density-dependent response was only slightly more pronounced among males compared with females (figure 1). This may be linked to the fact that the sexes have different growth priorities as males grow faster than females (Birgersson & Ekvall 1997), even when given a fixed quantity of food (Birgersson *et al.* 1998). As suggested by Birgersson *et al.* (1998), natural selection may have favoured a more efficient use of resources or food assimilation in males of dimorphic ungulates, allowing them to better compensate for developmental errors compared with females. However, more research is needed to investigate this idea. Despite the fact that females require less energy for growth (Ru *et al.* 2003), our findings suggest that their developmental stability is equally sensitive to environmental stress, perhaps because our study concerned juvenile deer for which sexual dimorphism is not yet marked; for example, jaw length (*infradentale–gonion caudale*) differed between the sexes by only 2.3% for fawns and 8.8% for yearlings during the moderate-density period. Our findings support earlier studies (Serrano *et al.* 2007) demonstrating plasticity of skeletal growth in response to environmental challenges among females also.

Contradicting our expectations, the ability to buffer density-dependent stresses was independent of body condition, as we observed no relationship between condition and individual CFA. Although a negative relationship between condition and FA of secondary sexual characters has been documented in cervids (Ditchkoff *et al.* 2001), there is little information on the relationship between FA of skeletal characters and body condition. In small mammals, for example, developmental instability of skeletal characters is unrelated to body condition (Badyaev *et al.* 2000). We suggest that when conditions are sufficiently limiting, all deer of a given cohort are unable to buffer against developmental error, irrespective of body condition. In conclusion, to explore further how FA of skeletal parts may be suitable for biomonitoring, more research is needed to elucidate better the relationship between FA in skeletal traits and phenotypic quality.

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Badyaev, A. V., Foresman, K. R. & Fernandes, M. V. 2000 Stress and developmental stability: vegetation removal causes increased fluctuating asymmetry in shrews. *Ecology* **81**, 336–345. (doi:10.2307/177431)

Birgersson, B. & Ekvall, K. 1997 Early growth in male and female fallow deer fawns. *Behav. Ecol.* **8**, 493–499. (doi:10.1093/beheco/8.5.493)

Birgersson, B., Tillbon, M. & Ekvall, K. 1998 Male-biased investment in fallow deer: an experimental study. *Anim. Behav.* **56**, 301–307. (doi:10.1006/anbe.1998.0783)

Bjorksten, T. A., Fowler, K. & Pomiankowski, A. 2000 What does sexual trait FA tell us about stress? *Trends Ecol. Evol.* **15**, 163–166. (doi:10.1016/S0169-5347(99)01788-7)

Burnham, K. P. & Anderson, D. R. 2002 *Model selection and multimodel inference: a practical information–theoretic approach*, 2nd edn. New York, NY: Springer.

Carranza, J., Alarcos, S., Sánchez-Prieto, C., Valencia, J. & Mateos, C. 2004 Disposable-soma senescence mediated by sexual selection in an ungulate. *Nature* **432**, 215–218. (doi:10.1038/nature03004)

Damuth, J. 1981 Population density and body size in mammals. *Nature* **290**, 699–700. (doi:10.1038/290699a0)

Ditchkoff, S. S., Lochmiller, R. L., Masters, R. E., Starry, W. R. & Leslie Jr, D. M. 2001 Does fluctuating asymmetry of antlers in white-tailed deer (*Odocoileus virginianus*) follow patterns predicted for sexually selected traits? *Proc. R. Soc. B* **268**, 891–898. (doi:10.1098/rspb.2001.1588)

Feldhamer, G. A., Farris-Renner, K. C. & Barker, C. M. 1988 *Dama dama*. *Mammal. Species* **317**, 1–8. (doi:10.2307/3504141)

Forchhammer, M. C., Clutton-Brock, T. H., Lindström, Å. & Albon, S. D. 2001 Climate and population density induce long-term cohort variation in a northern ungulate. *J. Anim. Ecol.* **70**, 721–729. (doi:10.1046/j.0021-8790.2001.00532.x)

Gordon, I. J., Illius, A. W. & Milne, J. D. 1996 Sources of variation in the foraging efficiency of grazing ruminants. *Funct. Ecol.* **10**, 219–226. (doi:10.2307/2389846)

Hoffmann, A. A. & Woods, R. E. 2003 Associating stress with developmental stability: problems and patterns. In *Developmental instability: causes and consequences* (ed. M. Polak), pp. 387–401. New York, NY: Oxford University Press.

Kaji, K., Koizumi, T. & Ohtaishi, N. 1988 Effects of resource limitation on the physical and reproductive condition of sika deer on Nakanoshima Island, Hokkaido. *Acta Theriol.* **33**, 187–208.

Leamy, L. J. & Klingenberg, C. P. 2005 The genetics and evolution of fluctuating asymmentry. *Annu. Rev. Ecol. Syst.* **36**, 1–21. (doi:10.1146/annurev.ecolsys.36.102003.152640)

Lens, L., Van Dongen, S., Kark, S., Talloen, W., Hens, L. & Matthysen, E. 2001 The use of bilateral asymmetry in ecology and conservation: concepts, developments, and prospects. In *Recent research developments in ecology* (ed. S. G. Pandalai), pp. 24–41. Trivandrum, India: Transworld Research Network.

Parsons, P. A. 1990 Fluctuating asymmetry: an epigenetic measure of stress. *Biol. Rev. Camb. Philos. Soc.* **65**, 131–145. (doi:10.1111/j.1469-185X.1990.tb01186.x)

R Development Core Team 2007 *A language and environment for statistical computing*, v. 2.5.1. Vienna, Austria: R Foundation for Statistical Computing. (http://www.R-project.org)

Ru, Y. J., Fischer, M., Glatz, P. C., Wyatt, S., Swanson, K. & Falkenberg, S. 2003 Forage intake and nutrient requirements of fallow weaner deer in southern Australia. *Asian Austral J. Anim.* **16**, 658–692.

Serrano, E., Angibault, J.-M., Cargnelutti, B. & Hewison, A. J. M. 2007 The effect of animal density on metacarpus development in captive fallow deer. *Small Rumin. Res.* **72**, 61–65. (doi:10.1016/j.smallrumres.2006.07.007)

Serrano, E., Alpizar-Jara, R., Morellet, N. & Hewison, A. J. M. 2008 A half a century of measuring ungulate body condition using indices: is it time for a change? *Eur. J. Wildl. Res.* (doi:10.1007/s10344-008-0194-7)

Stamps, J. A. 1993 Sexual size dimorphism in species with asymptotic growth. *Biol. J. Linn. Soc.* **50**, 123–145.

Stewart, K. M., Bowyer, R. T., Dick, B. L., Johnson, B. K. & Kie, J. G. 2005 Density-dependent effects on physical condition and reproduction in North American elk: an experimental test. *Oecologia* **143**, 85–93. (doi:10.1007/s00442-004-1785-y)