

DEVELOPMENTAL INSTABILITY AND THE ENVIRONMENT: WHY ARE SOME SPECIES OF SHREWS BETTER INDICATORS OF STRESS THAN OTHERS?

KERRY R. FORESMAN AND ALEXANDER V. BADYAEV

ABSTRACT

Developmental instability is used commonly to assess environmental conditions, but it is frequently observed that some traits, individuals, and species are better indicators of environmental stress than others. Herein we addressed the mechanisms behind this variation by examining stress-induced developmental instability in three coexisting species of soricid shrews. We examined jaw development in shrews born in areas of extensive logging. We found that for each species, the sensitivity to stress was dependent on co-existence with other shrew species; a species reaction to stress was stronger when other shrew species were present on the study plot. Within species, morphology of the sex which seems

to have longer period of growth was more sensitive to stress during development. Finally, on an individual level, sensitivity of different traits to stress was proportional to the degree of their functional integration (i.e., interrelationships due to involvement in the same function); stress-induced variation was confined mostly to functionally independent traits. Thus, variation in behavioral interactions among coexisting related species, duration of growth period, and degree of functional integration should be considered when examining the relationship between developmental instability and environmental change.

INTRODUCTION

Morphology of an organism is an outcome of internal developmental interactions and of selection exerted by the environment during growth (Fusco, 2001; Arthur, 2002). A convenient way to isolate the role of environmental and genetic factors in trait development and morphology is to analyze fluctuating asymmetry of a trait (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). Because both body sides of an individual share the same genome and nearly identical environment, fluctuating asymmetry results from small random perturbations in developmental processes that take place on the left and right sides of the body. Indeed numerous studies used fluctuating asymmetry to infer environmental stress during development (e.g., Badyaev, 1998; Brakefield, 1997; Lens and Van Dongen, 2000; Manning and Chamberlain, 1994; Pankakoski et al., 1992). However, it is frequently observed that the relationship between the degree of fluctuating asymmetry and the intensity of environmental stress varies widely among traits within an individual, among individuals within a species, and among spe-

cies (reviewed in Møller and Swaddle, 1997). Documentation of this variation calls into question the usefulness of fluctuating asymmetry as a measure of the effect environmental stress has on developmental stability with some authors suggesting that the size of the trait provides a more useful and consistent measure of environmental variation during ontogeny than does fluctuating asymmetry (Evans and Hatchwell, 1993; Hunt and Simmons, 1997; David et al., 1998; Bjorksten et al., 2000). Thus, understanding the mechanisms that give rise to differential developmental instability is crucial in interpreting the evolutionary and ecological importance of fluctuating asymmetry. Several factors can influence the relationship between environmental stress during ontogeny and fluctuating asymmetry of a fully-grown trait.

First, traits can vary in developmental associations with other traits. For example, 2 traits that originate from the same developmental precursor might share variation due to partitioning of the pre-

cursor. Similarly, variation can be transmitted directly from one part to another by induction or traits can covary because they respond to the same genetic or environmental factor. Regardless, developmentally and functionally integrated traits should show similar patterns of fluctuating asymmetry in response to environmental variation (Klingenberg, 2003). Moreover, direct and compensatory interactions among traits involved in similar functions or developmental pathways may reduce the developmental perturbation in each of the integrated traits (Badyaev and Foresman, 2005). Thus, traits of high functional importance might have greater developmental stability.

Secondly, traits may differ in duration or rate of growth. Longer growing traits may be more susceptible to environmental variation during ontogeny. Similarly, traits with more rapid growth may be more dependent on environmental variation during growth or might be less capable of compensatory growth. A particular case is intraspecies variation in growth patterns between sexes in sexually dimorphic species and among individuals of different life history strategies (Badyaev, 2002).

Thirdly, traits and species might differ in intensity of selection against developmental abnormalities. For example, exposure to stress often triggers activation of stress-buffering developmental mechanisms (Hoffmann and Parsons, 1997). Activation of such mechanisms can be costly in terms of energy and resources and, depending on the environmental and social context in which growth occurs, individuals and species might differ in their ability to maintain such mechanisms.

Herein we empirically examined the effects of morphological integration, species coexistence and growth duration on the relationship between fluctuating asymmetry and environmental stress in three closely related species of *Sorex* shrews. First, we examined species differences in sensitivity to environmental stress during growth. We examined if the presence of a similarly-sized shrew species (an assumed competitor; Neet and Hausser, 1990; Churchfield, 1991; Kirkland, 1991) influences a species response to stress. Secondly, we compared fluctuating asymmetry between individuals that differ in duration of growth. We examined the association between the extent of sexual dimorphism in size and sexual dimorphism in fluctuating asymmetry in each species. Finally, we examined the relationship between morphological integration of a trait and its fluctuating asymmetry for each of the shrew species.

Shrews are an ideal system in which to address these questions. Existing work on the anatomy of the shrew mandible (e.g., Kindahl, 1959; Dötsch, 1982) allowed us to assign a degree of integration to morphological traits involved in foraging. Previous work on the sensitivity of the shrew mandible to environmental conditions (Badyaev et al., 2000), allowed us to design an appropriate experiment for a study of environmentally induced stress. Shrews are short-lived (18 months) insectivores with nearly continuous prey-searching and foraging activity, thus morphological variation in mandible traits (especially in muscle attachments which determine bite force; Carraway and Verts, 1994) is closely associated with fitness and is under strong current selection (Badyaev et al., 2000).

METHODS

This study was conducted in June - July 1994 - 1996 on eight experimental plots (ca. 17 ha each) located on four study sites within 32 km of one another in the Swan River Valley of western Montana, U.S.A. Each study site contained control (untreated) plots and plots where overstory vegetation was removed by Plum Creek Timber Company. We designed our study within these plots to minimize effects of immigration and emigration following treatments (see Badyaev et al., 2000 for details of

experimental design and trapping schedules). The vegetation removal treatment creates highly stressful conditions for shrews, as evidenced by high increases in developmental instability of shrew embryos (manifested as greater asymmetry in development), and by a decrease in individual condition of adults (Badyaev et al. 2000). We examined 560 two-three month old individuals of 3 closely related *Sorex* species (Fumagalli et al., 1999; see Foresman, 2001 for details of species biology): *Sorex cinereus*

control: 46 males and 43 females, vegetation removal: 74 males and 44 females; *Sorex monticolus* control: 37 males and 37 females, vegetation removal: 39 males and 38 females; *Sorex vagrans* control: 46 males and 52 females, vegetation removal: 46 males and 58 females. For some analyses, sex-related variation was removed in general linear models.

Left and right shrew mandibles were positioned on a slide and then photographed under 7.5X magnification using an Olympic SZH stereo photomicroscope and a video capture board. The resulting images were further magnified 2X in Mocha Image Analysis software (Jandel Scientific, San Rafael, California, USA). Data were x and y coordinates of 17 homologous morphological landmarks (Fig. 1; see Badyaev and Foresman, 2000 for a detailed discussion of these methods and error assessment). As a measure of the overall size of each mandible, we computed centroid size, which is the square root of the sum of squared distances of each landmark from the centroid (center of gravity) of the configuration (e.g., Dryden and Mardia, 1998). For centroid size,

the analyses of variation among individuals and of fluctuating asymmetry in offspring of females that underwent pregnancy under stressful conditions were conducted using standard univariate statistics (Palmer and Strobeck, 1986).

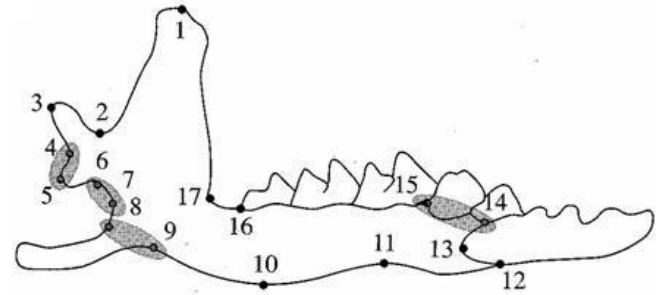


Fig. 1. – Shrew (*Sorex*) mandible showing the position of 17 landmark points used in this study. Traits (M- in “muscle” area, and T- in “teeth” area) were as follows: M12 1-2, M19 1-9, M23 2-3, M34 3-4, M45 4-5, M46 4-6, M58 5-8, M817 8-17, T1013 10-13, T1016 10-16, T1115 11-15, T1116 11-16, T1213 12-13, and T1416 14-16. Shaded regions reflect functionally integrated sites for muscle attachment.

RESULTS AND DISCUSSION

SPECIES VARIATION

To examine the effect of species coexistence we first compared size of the coexisting species. We found that the centroid size of the jaw (in mm²) was significantly smaller in *S. cinereus* [638.91 ± 24.97(SE)] compared to either *S. monticolus* (840.40 ± 50.35) or *S. vagrans* (832.301 ± 48.03; difference between species: $F = 800.2$, $p = 0.0001$; Fig. 2A.). However *S. monticolus* and *S. vagrans* were not different from each other regardless of their co-occurrence on the same plot ($F_{\text{plot}} = 1.31$, $p = 0.4$, $F_{\text{species}} = 0.58$, $p = 0.9$). Thus, similarly-sized *S. monticolus* and *S. vagrans* are most likely to compete for similar resources and, competition likely is to be more intense under experimental stress.

We then compared developmental instability of individuals that coexisted with similarly-sized individuals of different species. We compared fluctuating asymmetry in jaw centroid for *S. monticolus* following the treatment between the plots where *S.*

monticolus co-occurred with *S. vagrans* and the plots where only one species was present. The sensitivity to stress depended upon coexistence with similarly-sized species in the shrew communities. We found significantly higher levels of fluctuating asymmetry in *S. monticolus* in plots where *S. vagrans* was present during stressful treatment ($F_{\text{treatment} \times \text{coexistence}} = 26.1$, $p < 0.0001$; Fig. 2B). Fluctuating asymmetry in centroid size (0, SD; unsigned left minus right side values and adjusted for directional asymmetry) was as follows: *S. monticolus* following treatment in plots with *S. vagrans* present - before treatment: 9.13 (10.88), after treatment: 35.52 (17.62); $t = -5.13$, $p = 0.0001$; *S. monticolus* following treatment with *S. vagrans* absent: before treatment: 12.89 (15.94), after treatment: 24.92 (15.10), $t = -1.33$; $p = 0.09$.

The relationship between species coexistence and response to stress may be mediated by greater competition for shared food resources or increased predation under treatment conditions. In shrews,

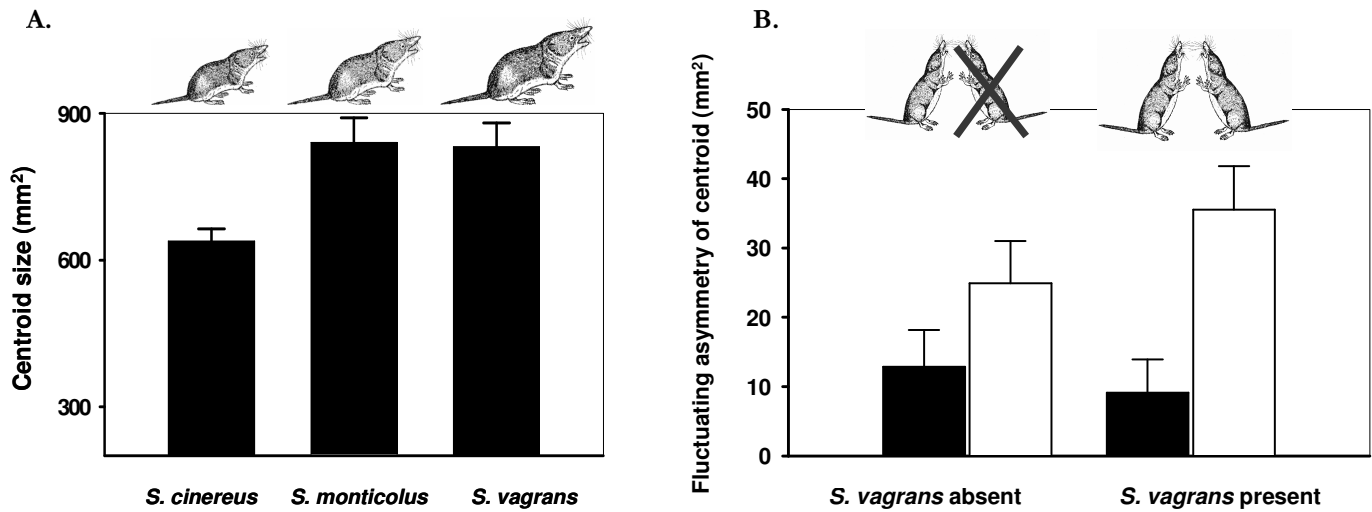


Fig. 2. – Species variation in A, centroid size, and B, degree of fluctuating asymmetry in the presence and absence of similar-sized species before (solid bars) and after (open bars) treatment (mean ± SE).

periods of intense environmental stress often are associated with increased food competition caused by major habitat alterations (e.g. Badyaev et al., 2000 and references therein), or extreme population fluctuations (e.g. Zakharov et al. 1991). Stress-induced modifications of shrew mandible traits may be beneficial if resulting differences in bite force or chewing patterns allow consumption of new prey items (Carraway and Verts, 1994).

Because most of the variation in developmental stability in skeletal characters arises during prenatal and early postnatal stages (e.g., Siegel and Doyle, 1975), physiological condition of pregnant females could be a proximate cause of variation in offspring asymmetry. Similarly, Zakharov et al. (1991) found that physiological conditions of females and developmental stability of their offspring strongly differed between geographic populations in *Sorex araneus* and were concordant with fluctuations in population density. Frequent territorial disputes, increased resource competition, and increases in predator numbers accounted for increased stress in shrews during peak population densities (Zakharov et al., 1991).

SEX VARIATION

We compared differences in jaw centroid size between sexes of each species to account for any

size differences related to sex. Species differed in sexual dimorphism in jaw centroid size (F species x sex = 27.3, $p = 0.001$). Under control conditions, *S. vagrans* had the highest levels of sexual dimorphism, followed by *S. monticolus* and *S. cinereus* (Fig. 3A). *S. vagrans* - male size: 854.58 (53.40), female size: 819.13 (32.19); $t = 3.27$, $p = 0.02$; *S. monticolus* - male size: 835.68 (16.69), female size: 805.73 (41.33), $t = 2.19$, $p = 0.05$; *S. cinereus*: males 638.20 (18.14), females: 634.12 (4.96), $t = 0.38$; $p = 0.70$.

The degree of stress response was proportional to the extent of sexual dimorphism in size (Fig. 3B); species with larger size dimorphism had larger differences between sexes in response to stress. Percent of total variance (calculated from variance components) in fluctuating asymmetry response following treatment that was due to sex differences in centroid size was as follows: *S. vagrans* 72.2%, *S. monticolus* 54.1%, and *S. cinereus* 31.2%; F sex x species = 47.9, $p = 0.001$).

The larger sex had a greater increase in asymmetry in response to treatment. This may be due to its longer exposure to stress or stronger directional selection for faster, but less buffered growth in the larger sex (Møller and Pomiankowski, 1993). Elsewhere, we have shown that mandibular traits that were most strongly affected by stress are late-maturing traits (Badyaev, 2002). Other factors,

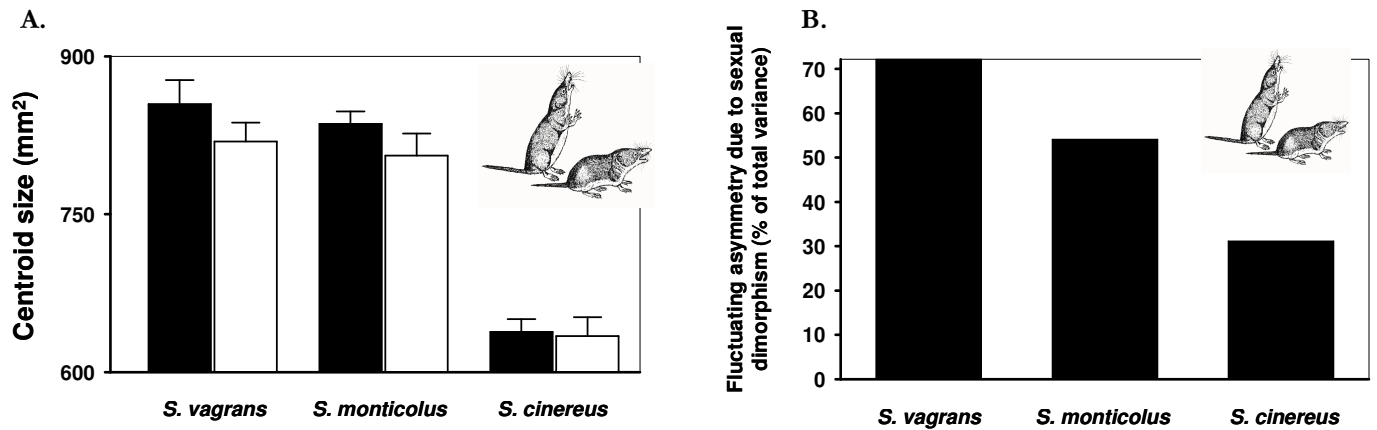


Fig. 3. – Sexual variation between species in A, overall centroid size (solid bars = males; open bars = females), and in B, degree of fluctuating asymmetry following stress (mean \pm SE).

untested here, may also influence such responses, e.g., variable parasite loads, sex-related hormone profiles.

The mating system of most *Sorex* shrews is poorly known, but in *S. araneus* males intensively compete for access to females during an extremely short (2 hours) estrus period (Stockley et al., 1996). In this species, the timing and intensity of growth appears to be more variable in males than in females, resulting in different mate-searching strategies used by early- and late-maturing males (Stockley et al., 1994). Males that are larger at early stages of sexual maturation had large home ranges in areas with high female density, whereas smaller males had smaller home ranges and made long-distance movements to visit female ranges (Stockley et al., 1994). Thus, there may be stronger selection on males to reach adult size sooner and to establish a breeding range; the faster, shorter, and more variable growth of males may cause them to be more sensitive to stress than are females.

TRAIT VARIATION

In all three species, interlandmark distances differed in response to stress (F 's landmark > 254.0 , $p < 0.0001$; Fig. 4A). Percent of total variance (calculated from variance components) due to fluctuating asymmetry varied among landmarks from 48.5 to 92% in *S. cinereus*; 31.2 - 78.8% in *S. monticolus*, and 28.3 - 54.3% in *S. vagrans*. We calculated integration

among interlandmark distances with Kendall's coefficients of concordance and found that in all three species, traits with the greater degree of morphological integration (inter-relationships with other traits) had lower fluctuating asymmetry following treatment compared to less integrated traits (all Sherman's r 's > 0.5 , p 's < 0.05) (Fig. 4B). Traits closely related to individual fitness are likely to be more integrated than other traits, thus may have the lowest sensitivity to environmental variation.

Low environmental canalization of non-integrated traits may be associated (either directly or indirectly) with low genetic canalization of these traits (Gavrilets and Hastings, 1994; Wagner et al., 1997). Thus, non-integrated traits may be highly sensitive not only to environmental, but also to genetic pressures (such as selection and genetic drift). Mandibular traits strongly differed in their sensitivity to environmental conditions. The differential sensitivity of trait development to environmental change is assumed to be directly related to the intensity of stabilizing selection (e.g., Schmalhausen, 1949; Mather, 1953; Soulé and Cuzin-Roudy, 1982; Kieser, 1987). Traits of high functional importance, that are usually under stronger stabilizing selection, become highly canalized and are developmentally more buffered than less functionally important traits (Mather 1953; Kieser 1987; Gavrilets and Hastings, 1994; Stearns and Kawecki, 1994; Pomiankowski and Møller, 1995). However, the developmental

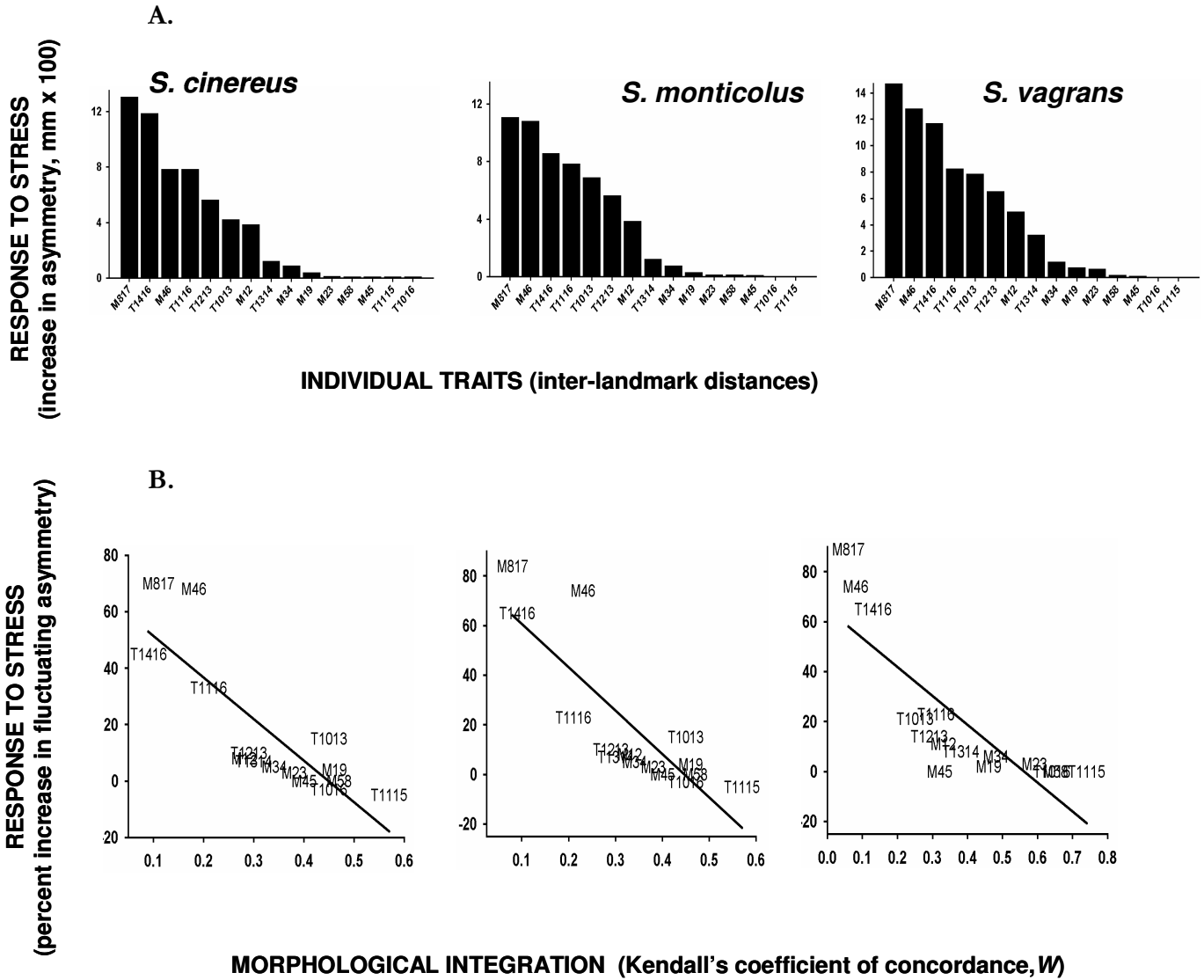


Fig. 4. – Mandibular trait variation in species: sensitivity to stress: A, individual traits, and B, proportional response to degree of morphological integration.

mechanisms that ultimately control differential developmental instability are poorly understood (e.g., Emlen et al., 1993; Moreno, 1994 and references therein; Swaddle and Witter, 1997). Our results indicate that high morphological integration is closely

associated with low stress-induced variation, and traits that show the smallest response to stress also were more closely related to individual fitness (Badyaev and Foresman, 2000).

ACKNOWLEDGMENTS

We thank R. McGraw and G. Parkhurst for collection of shrews, and C. Fiumara and M. Fer-

mandes for carefully digitizing thousands of shrew mandibles.

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