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Developmental stability in geophilomorph centipedes

Abstract: Measures of fluctuating asymmetry, the random and not inheritable deviations from perfect bilateral symmetry, are routinely used as indices of developmental stability. The almost homonomous trunk of geophilomorph centipedes presents another kind of symmetry: the series of segments are arranged according to a *translational symmetry*. We found that the segmental pattern of some metric characters presents high levels of local disparity between contiguous segments, these irregularities resembling the effect of developmental noise affecting a more regular underlying pattern. We present a preliminary study of the developmental stability of geophilomorph segment differentiation, dealing with segmental patterns of metric characters in the trunk of two species. We found good correlation between the developmental stability in segmental differentiation expressed as translational asymmetry (TA) and the degree of developmental stability estimated by measures of traditional (bilateral) fluctuating asymmetry (FA).

Key words: Developmental stability, fluctuating asymmetry, segmentation, segmental pattern

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INTRODUCTION

In recent time, centipedes have appeared on the stage of theoretical evolutionary biology. The puzzling regularities of their segment numbers (MINELLI & BORTOLETTO 1988) and of their segmental patterning (BERTO *et al.* 1997) elicited interesting questions in the field of genetics and evolution of segmentation. Millipede and centipede morphology stimulated a revisitation of the controversial concept of homology (MINELLI 1996). More recently, centipedes have been used as model system for the study of macroevolutionary trends of morphological complexity (FUSCO & MINELLI

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2000) and as emblematic taxon in the foundation of the emerging discipline of evolutionary developmental biology (ARTHUR 1999).

In this study we will show that geophilomorph centipedes offer also special opportunities for the study of morphological evolution through the analysis of developmental stability.

Recent theoretical studies in evolutionary biology insist on presenting evolution as the change of ontogenies with time (GILBERT *et al.* 1996). Unfortunately, the relationship between genotype and phenotype through development is poorly known but for a few species, the so called model systems. On the other hand, accurate study of an organism's morphology, concentrating on the *performances* of the developmental system, can provide useful information on its structure, even if we ignore its genetic constitution and epigenetic history.

Developmental stability is the production of consistent phenotypes under a range of environmental and genetic conditions (MØLLER & SWADDLE 1997). Developmental stability is a property of the developmental system reflecting the organism's ability to resist deleterious effects of perturbation during development (developmental noise). Measures of fluctuating asymmetry, the random and not inheritable deviations from perfect bilateral symmetry (VAN VALEN 1962), are routinely used as indices of developmental stability because the two sides of a bilaterally symmetric organism share the same genome and environmental differences between the two sides are likely to be small.

For a geometrical object, symmetry can be defined as a geometric transformation that does not produce any variation, i.e. an object that has certain symmetries is invariant under those transformations. Common kinds of symmetry for living organisms include bilateral symmetry, translational symmetry and radial symmetry. For an organism, the specific symmetries of its bauplan can be conceived of as an idealized outcome of unperturbed development. Therefore, any kind of deviation from symmetry, detectable as morphological irregularity, can potentially be exploited for studying developmental stability (FREEMAN *et al.* 1993).

The almost homonomous trunk of geophilomorph centipedes presents a series of repetitive structures, the segments, arranged according to a *translational symmetry*. This symmetry is not perfect, not even in an ideal animal developing without disturbances: the ith segment is not expected to be the exact copy of the i-1th segment, just shifted caudally one segmental position. Nevertheless, we found that the segmental pattern of some metric characters presents a high level of local disparity between contiguous segments, these irregularities resembling random perturbations on a basic pattern (Fig. 1). Individual variation in degree and kind of irregularity cannot be explained as a population level polymorphism affecting segment specification. Developmental noise, probably reflecting developmental instability, seems to be a better candidate. This instability, expressed as deviation from the specimen's ideal segmental pattern, can be detected even if the underlying segmental pattern is not a straight line with slope zero (a perfectly invariant segmental series) but, instead, a curve.

We present a preliminary study on the developmental stability of geophilomorph segment differentiation, investigating segmental patterns of metric characters in the trunk of two species. Our morphometric analysis is specifically devised for evaluating developmental stability in segmented animals. It considers within-specimen local irregularity of segments as well as a measure of morphological irregularity at the level of the whole specimen. We compare the developmental stability in segmental differentiation detected as irregularities of the segmental pattern (translational asymmetry, TA) with the degree of developmental stability estimated by measures of traditional (bilateral) fluctuating asymmetry (FA).

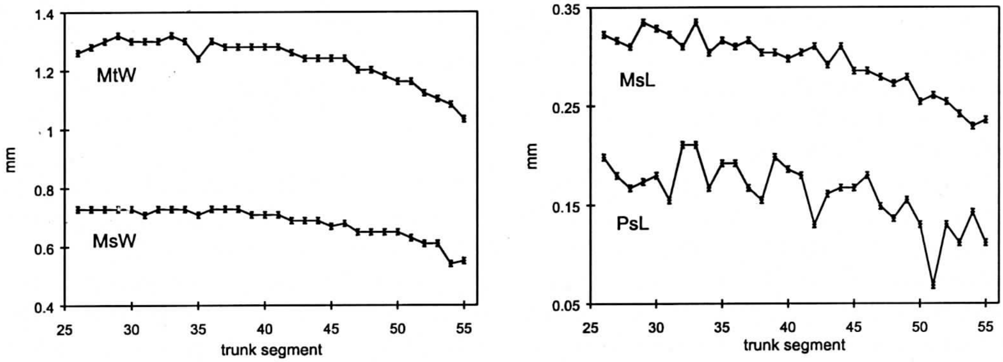

Fig. 1. Segmental pattern of four metric characters in the trunk of a single specimen of *Pleurogeophilus mediterraneus*. Note the different irregularity of the patterns. Bars are measurement precision. Abbreviations, see legend to Fig. 2.

MATERIAL AND METHODS

Material

This study is based on five male specimens of *Pleurogeophilus mediterraneus* (MEINERT, 1870) with 61 leg-bearing segments and five male specimens of *Strigamia acuminata* (LEACH, 1815) with 39 leg-bearing segments. The specimens, collected in different years in woodlands of the Venetian Prealps (Northern Italy), are preserved in A. Minelli's collection (University of Padova, Italy).

Cuticle preparations

For improving measurement precision, a special preparation of the cuticle preceded the morphometric analysis of the material. Exuvia-like preparations were obtained from specimens stored in 70% ethanol by proteolytic digestion, modified from HILKEN's (1994) protocol. The specimens were incubated in 50 mM $(NH_4)_2CO_3$ adjusted to pH 8 with Na_2CO_3 for 24 hours at 37°C, then digested in trypsin (1 mg/ml) in the same solution at 37°C for 2 to 7 days, depending on body size. After complete digestion, the remaining cuticle was washed in water and stored in 70% ethanol. Exuvia-like preparations of the trunk, longitudinally cut along the pleural region with fine scissors to separate the array of dorsal sclerites from the ventral one, were mounted on ordinary glass slides with glycerol.

Characters and measures

Eight metric characters of trunk segments were selected to study the developmental stability of segmental patterns (Fig. 2). These characters, with their code, are the following:

MsL/MsR	distance between two idionymic setae on the left/right margin of metasternite
MsW	width of metasternite, measured as the distance between two idionymic setae on the left and right margins
PsL/PsR	distance between two idionymic setae on the left/right presternite
MtL/MtR	distance between two idionymic setae on the left/right margin of metatergite
MtW	width of metatergite, measured as the distance between two idionymic setae on the left and right margins

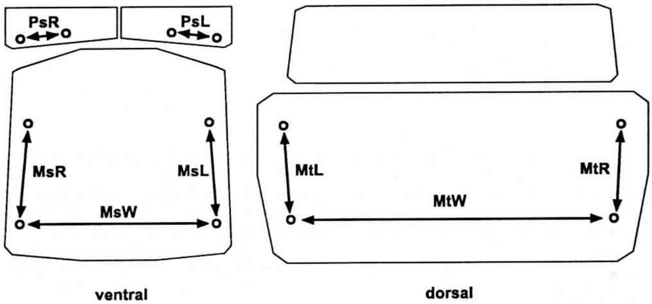

Fig. 2. Schematic representation of the ventral and dorsal sclerites of a geophilomorph trunk segment illustrating the characters used in morphometric analysis. Circles represent idionymic setae. MsL/MsR, distance between two idionymic setae on the left/right margin of metasternite; MsW, width of metasternite, measured as the distance between two idionymic setae on the left and right margins; PsL/PsR, distance between two idionymic setae on the left/right presternite; MtL/MtR, distance between two idionymic setae on the left/right margin of metatergite; MtW, width of metatergite, measured as the distance between two idionymic setae on the left and right margins.

As morphological landmarks we preferred some idionymic setae (i.e. setae individually distinguishable from the others and recognizable as homologous in different specimens) to the more usual points along sclerite margins, in order to avoid a measurement error so large as to conceal any real segmental irregularity. As a matter of fact, sclerite margins in geophilomorphs are often poorly defined (*cf* MANTON 1965). With a micrometer mounted on a microscope, measurement were made to the nearest micrometer unit (1 unit = 6.2 µm for MsL, MsR, MsW (in *S. acuminata*), PsL, PsR, MtL and MtR; 1 unit = 19.7 µm for MsW (in *P. mediterraneus*) and MtW).

Measures of MsL and MsR vs. MsW, and MtL and MtR vs. MtW are not entirely independent because they share some landmarks (Fig. 2). But because of the magnitude of MsL and MsR variation in respect to MsW, and the magnitude of MtL and MtR variation in respect to MtW, and because longitudinal and transversal measures have almost orthogonal orientation on the sclerites, the bias is smaller than the measurement error.

For each specimen, the eight measures listed above were repeated in 30 trunk segments (XXVI to LV) in *P. mediterraneus* and in 20 trunk segments (XVI to XXXV) in

S. acuminata. The different sets of segments chosen in the two species correspond to the same region of the trunk, considering the different overall segment numbers in the two species and the relative position of the segments in the trunk. Morphometric analysis was concentrated on this limited section of the trunk for two reasons: a) preliminary analyses showed that, in this region, the species-specific segmental pattern, for metric characters, tends to be a simple curve (if not a straight line): this simplified the correction for the deviation from a perfect translational symmetry; b) in a limited region, segments are more similar each other, increasing the degree of serial homology for the characters measured.

Morphometric analysis

For each segment, three measures of FA, corresponding to the three pair of bilateral characters (MsR – MsL, PsR – PsL, MtR – MtL) were calculated as

$$\frac{R_i - L_i}{\frac{1}{2}(R_i + L_i)}$$

where R_i and L_i are respectively the right and the left measurements of the character in the *i*th element of the segmental series. Specimen measures of FA were calculated as the average of the *N* unsigned (absolute) values of FA of the segmental series (N = 30 for *P. mediterraneus*, N = 20 for *S. acuminata*). These are standard, size corrected indices of FA (PALMER & STROBECK 1986).

For each segment, eight size corrected measures of TA, corresponding to as many characters, were calculated as

$$\frac{X_i - Y_i}{Y_i}$$

where X_i is the measurement taken from the *i*th element of the series and Y_i is the value of the *i*th element predicted by a second degree polynomial fitting of the segmental series (specific for specimen and character). Specimen measures of TA were calculated as the average of the *N* unsigned (absolute) values of TA of the segmental series. A similar approach was used by FREEMAN *et al.* (1993) in a study of TA of leaves of the flowering plant *Campanula arvensis*.

In a small sample we tested the precision of character measurement by repeated independent measures of the same traits. Measurements are highly repeatable (because setae are neat landmarks), their average variation (standard deviation) being smaller than the precision of our ocular micrometer. Therefore we considered each measurement as a uniform random variable centred on the measure and with a range equal to the micrometer precision. The error for a single value of FA or TA, relative for example to one character in one segment of a single specimen, was calculated applying the standard formulas for error propagation in compound measures. When several FA and TA values are averaged, 95% confidence intervals were calculated considering the variance of the sum of different independent uniform random variables (the single-segment FA and TA measures).

Standard correlation analysis was performed between different measures of FA and TA, both relatively to individual segments within specimens and at the level of whole specimens. We looked for within-specimen correlation among the three measures of FA and for correlation between dorsal and ventral TA of corresponding characters i.e. MsL vs. MtL, MsR vs. MtR and MsW vs. MtW.

This approach is primarily intended to detect within-specimen differences in developmental stability, therefore our small samples do not allow to extend the result of our analysis to a comparison between the two species. Not all the statistics of the two samples (five specimens each) can be considered representative of the relative population. Therefore error bars shown in the figures consider exclusively the uncertainty due to measurement error and not sample error.

RESULTS

Degrees of irregularity in different characters

All specimens in both species present the same arrangement of FA and TA (Fig. 3). The presternite is much less stable then metatergite and metasternite, this last sclerite presenting the lowest level of irregularity. Measures of sclerite width (MsW and MtW) present TA close to zero. Differences between the two species are negligible.

Analysis of segmental patterns of instability

We found no species-specific segmental position with exceptional instability. Neither did we find clues of any periodicity in FA and TA segmental pattern. A slight antero-posterior increase in instability is perhaps observable in *P. mediterraneus* (Fig. 4).

We found significant correlation between segment instability expressed by FA and instability expressed by TA ($r = 0.82$, $n = 30$, $p < 0.01$ for *P. mediterraneus*; $r = 0.74$, $n = 20$, $p < 0.01$ for *S. acuminata*; Fig. 5). The correlation is significant even within specimens (r ranging between 0.50 and 0.78, $p < 0.05$) but for two specimens of *S. acuminata* ($r = 0.40$, $r = 0.17$).

Correlations between segment measures of instability within specimens

We found non significant ($p > 0.1$) within specimen correlations among signed FA measurements in both species but for two specimens of *P. mediterraneus* which present a weak correlation between metasternite and presternite FA ($r = 0.43$, $n = 30$, $p < 0.05$, and $r = 0.38$, $n = 30$, $p < 0.05$).

As for correlation between dorsal and ventral TA, in *P. mediterraneus* we found five cases (out of 15) of significant correlation ($P < 0.05$), i.e. 3 cases of correlation between MsW and MtW and 2 of correlation between MsL and MtL. In *S. acuminata* we found two cases of significant correlation ($p < 0.05$) (again, correlations between

MsW and MtW) with some other cases of correlation at a lower level of significance (5 cases, $p < 0.1$).

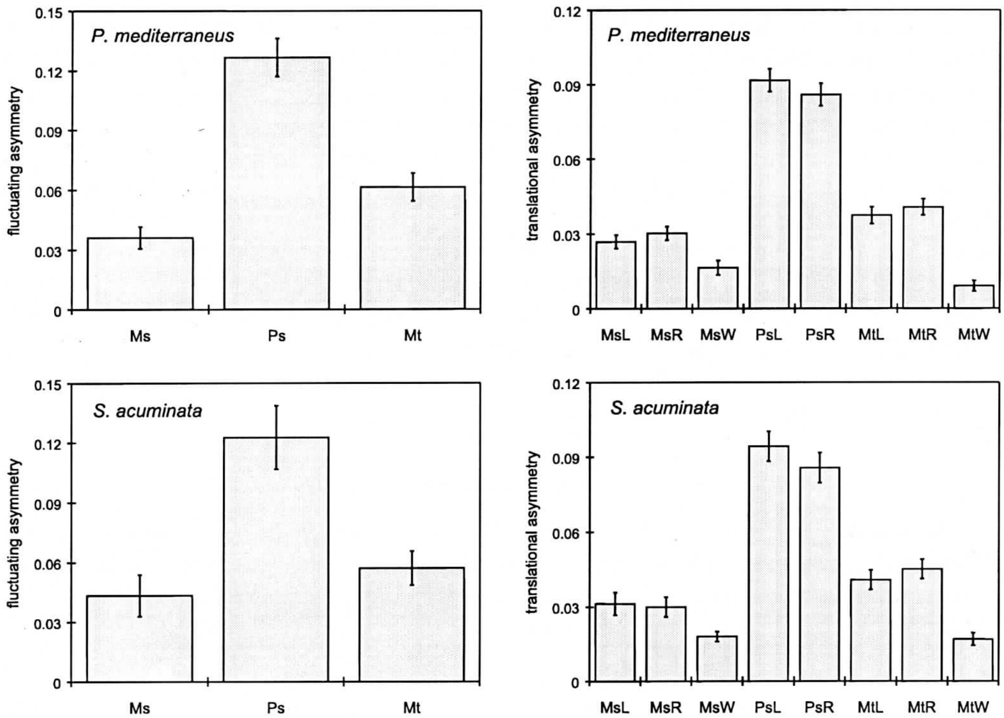

Fig. 3. Diagrams illustrating specimen fluctuating (left) and translational (right) asymmetry exhibited by different characters. Values of one species are obtained averaging the values of specimen FA and TA of the five specimens of the corresponding sample. Bars represent averages of the five within-specimen 95% confidence intervals. Abbreviations, see legend to Fig. 2.

DISCUSSION

Different characters show different degrees of segmental instability and the pretergites present a very high level of FA and TA. It is important to note that these results do not depend on the choice of idionymic setae as character landmarks. TA values for MsW and MtW are very low. Even a coarser measure than the distance between idionymic setae could capture the same: the irregularity we recorded for PsL and PsR would have been recorded also from width measured as distances between sclerite margins. For instance, in *P. mediterraneus*, in the first third of the trunk each hemipresternite is divided in two sclerites, the suture between them laying laterally of the more lateral seta we considered. Moving caudally: a) the suture of the two sclerites tends to became a furrow or to disappear; b) the position of the suture/furrow moves medially to the zone between the two setae, which often maintain their position relatively to the other sclerites. But this transition is not uniform and it also does not develop in parallel at the two sides of the body! In the sequence of segments, independently at the two body sides, the hemipresternite suture moves medially and laterally with no correlation with the segmental irregular variation in its depth (distinctness). To a lesser degree, a similar unusual behaviour is also observable in the presternites of *S. acuminata*.

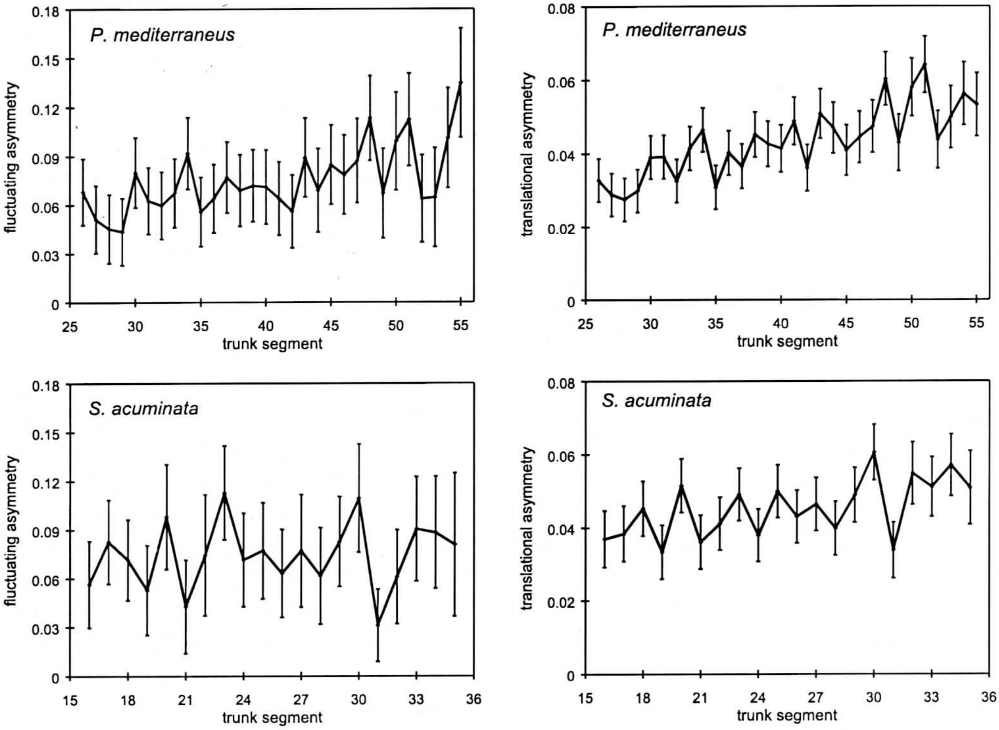

Fig. 4. Segmental pattern of fluctuating (left) and translational (right) asymmetry in the two species samples. For each species, the values of FA end TA for each segment are the averages of the five specimen FA and TA for that segment. For each segment there are three measures of FA and eight measures of TA. Bars represent averages of the five within-specimen 95% confidence intervals.

The generally non significant correlation between FA of different characters is not surprising. Other studies found that FA of two traits correlate very minimally with each other within a population (see MØLLER & SWADDLE 1997). It has been suggested that this weak correlation could be due to the traits being affected by different levels of stress, or to the different buffering abilities of different developing structures, or even to distinct times of sensitivity to the developmental noise. More recently, GANGESTAD & THORNHILL (1999) presented a mathematical model of the relationship between developmental precision and FA that predicts weak correlation between FA of different traits without introducing trait-specific differences in developmental stability. The theoretical debate is still open. However, our within-specimen weak correlations are difficult to explain in the same frame of within-population studies. A further study of the ontogeny of FA will probably clarify the relation between developmental buffering and external noise during growth (see below).

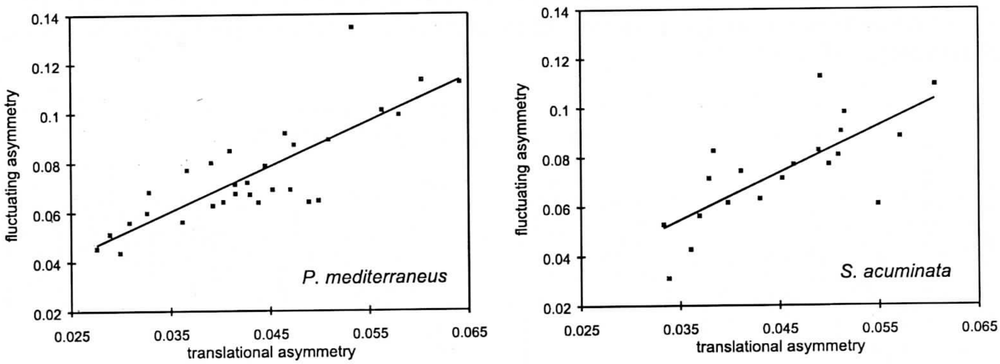

Fig. 5. Scatter plots of the relationships between fluctuating asymmetry and translational asymmetry in the two species samples. Regression lines are shown.

We found a slightly better, but still weak, correlation between TA of paired dorsal and ventral characters. This suggest that the developmental noise recorded by this morphological irregularity affected the morphogenesis of segmental patterning rather than the process of segmentation itself. Otherwise, a stronger correlation between different characters in their deviation from the normal pattern would be expected.

Theoretically, it is possible to generate segmental patterns with high TA and low FA (for instance, with translational irregularity affecting concordantly right and left traits) or, at the opposite, high FA and low TA (for instance, with signed bilateral asymmetry equal in all segments). Other, more general, kinds of segmental irregularities result in disturbing simultaneously bilateral and translational symmetry. The good correlation between measures of segmental instability detected by FA and TA suggest that this last kind of segmental instability is more frequent than the previous ones. Apparently, FA and TA, extended over several characters, detect the same traces of segmental instability.

The agreement between the two approaches encourages the use of TA in the study of developmental stability of segmented animals along with the more studied and standardized FA procedures. TA, working on a different axis of symmetry, is potentially able to reveal developmental noises that do not affect bilateral symmetry. A possible drawback is that a preliminary analysis of the segmental pattern is necessary for correcting the data for the commonly occurring imperfections of translational symmetry.

For the future, beyond extending this approach to larger samples of different species, it would be very interesting to investigate the ontogeny of segmental instability via longitudinal analysis, i.e. following the development of asymmetries, moult after moult, in subsequent developmental stages in the same individual as in a recent study (TOMKINS 1999) on the forceps of earwigs. This could offer interesting information on the interdependence of growth trajectories of different traits and on the role (if any, see APARICIO 1998) of compensatory growth between left and right sides for buffering developmental noise. A study of the interplay between different mechanism of developmental buffering would be of special interest in animals like centipedes that, during the germ band stage, develop as two separate halves which longitudinally fuse only at a later stage (HEYMONS 1901).

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