On the ontogeny of developmental stability in a stabilized trait

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SUMMARY

Developmental stability is most accurately assessed by measuring the small asymmetries between left and right elements of bilateral traits, i.e. fluctuating asymmetry. Although there has been much recent interest in fluctuating asymmetry, as low asymmetry may reflect high fitness, relatively little is known concerning the developmental origins of these minor discrepancies. Understanding the mechanisms that determine fluctuating asymmetry is crucial to interpreting much of the recent literature, for example, it has often been claimed that asymmetry reflects properties of an individual's genome. Therefore, in this study, we have examined the ontogeny of fluctuating asymmetry in the primary feathers of European starlings (*Sturnus vulgaris*) and compared our data with six published hypotheses of the mechanisms of asymmetry development. We found that signed asymmetries were not consistently biased toward either the left or the right side among feathers on the same individual; growth increments (measured every two days) were also not sided; and both absolute and relative asymmetry decrease as the feathers develop. These data are most consistent with a developmental regulatory system that involves some elements of feedback between left and right sides and episodes of compensational growth to correct large asymmetries. We discuss the possible differences in developmental mechanisms that determine asymmetry in traits of varying functional importance.

1. INTRODUCTION

Developmental stability can be accurately assessed by comparison of left and right elements of bilaterally symmetrical traits (Ludwig 1932; Beardmore 1960; Waddington 1960). The minor discrepancy between left and right sides, fluctuating asymmetry, results from both genetic and environmental factors (see reviews in Palmer & Strobeck (1986); Møller & Swaddle (1997)). There is evidence to indicate that individuals with increased developmental stability (i.e. low asymmetry) may have increased individual fitness (see reviews in Møller & Pomiankowski (1993); Watson & Thornhill (1994); Møller & Swaddle (1997)) and developmental stability can be selected for through sexual and natural selection processes (e.g. Møller 1992, 1993; Balmford *et al.* 1993; Evans & Hatchwell 1993; Swaddle & Cuthill 1994; Allen & Simmons 1996; Bennett *et al.* 1996; Swaddle 1996). Therefore, there has been much interest in fluctuating asymmetry in the recent evolutionary literature.

However, the processes by which fluctuating asymmetries develop have received relatively little attention. Investigations into the mechanisms that give rise to developmental instability (and hence asymmetry) are crucial to understanding the role that asymmetry plays in evolutionary processes, and may further reveal the situations under which asymmetries occur. It is often difficult to fully interpret the importance of asymmetry studies without understanding the mechanisms that give rise to the asymmetric phenotype. At present, there are at least six non-mutually exclusive hypotheses that have been suggested to describe the ontogeny of fluctuating asymmetry: (i) directional external cues; (ii) 'coin-toss' hypothesis; (iii) magnification of asymmetry; (iv) accumulation of accidents; (v) persistent asymmetries; and (vi) compensational growth. These hypotheses are described below.

The first hypothesis suggests that directional (or sided) environmental influences could lead to asymmetric development (Gruneberg 1935). In such a case, we would expect asymmetry to be consistently biased toward one side of an organism; but at the population level, asymmetry could be non-directional (Hallgrímsson 1993). For instance, if individuals were oriented in different directions (with respect to left and right sides) but the environmental perturbation emanated from a single, fixed location. Such directional cues are likely to be rare in motile organisms, as individuals may have the ability to change the direction of the external cue relative to the body.

Second, the 'coin-toss' hypothesis suggests that morphogenesis is composed of independent developmental units within which the directionality of an inherent level of asymmetry is randomly assigned (cf. Soulé 1982; Hallgrímsson 1993). This hypothesis predicts that as the number of developmental units (coin tosses) increases, relative asymmetry will de-

Proc. R. Soc. Lond. B (1997) **264**, 329–334
Printed in Great Britain

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Table 1. *Predictions generated by the six hypotheses for the ontogeny of fluctuating asymmetry and empirical evidence indicated by growth of starling primary feathers. Responses in bold indicate that they are supported by the starling primary feather data.*

questions	hypotheses						data primary feathers
	directional external cues	coin toss	magnification of asymmetry	accumulation of accidents	persistent asymmetries	compensational growth	
within individuals, are final primary feather lengths on one side consistently larger than on the other?	yes	**no**	**no**	**no**	**no**	**no**	no
at each developmental stage, is growth biased toward one side?	yes	**no**	yes	**no**	yes	**no**	no
does asymmetry accumulate during the development of a trait?	yes	**no** (opposite for relative asymmetry)	yes	yes (but only at later growth stages)	**no** (remains constant)	**no** (opposite for relative and absolute asymmetry)	no (opposite for relative and absolute asymmetry)

crease. If developmental units are positively associated with size, then we would expect trait size and relative asymmetry to be negatively related.

Third, it is has been suggested that a small, random bias early in development can be magnified during subsequent morphogenesis to lead to a larger final asymmetry. The asymmetry increments could be constant during development, but are necessarily biased to one side. Developmental units are therefore non-independent (e.g. Emlen *et al.* 1993; Graham *et al.* 1993; Hallgrímsson 1993). Gruneberg (1935) has proposed that such an asymmetry could occur as a result of the slight differences in physicochemical conditions of the cytoplasm on either side of the body. Presumably, this would not possess any directionality across a group of individuals as the differences are randomly determined and, therefore, the asymmetry would be non-directional at the population level.

The fourth hypothesis suggests that no trait follows an ideal developmental pathway and developmental noise accumulates over time at a level below morphogenesis (e.g. Waddington 1958; Hallgrímsson 1993). Therefore, the time taken to develop a trait should be positively related to fluctuating asymmetry (but not necessarily the size of the trait) as the accumulated development noise will be greater. Presumably, as developmental noise accumulates at a level before morphogenesis, asymmetry only becomes manifest at later stages of growth, hence asymmetry will tend to increase over time during the development of a given trait.

Fifth, the size and direction of asymmetries are determined early in ontogeny and hence the sign and magnitude of asymmetries persist at all growth stages (Chippindale & Palmer 1993). This hypothesis predicts that asymmetries do not alter among growth stages; but asymmetries can differ in direction among traits as the magnitude and direction of asymmetry are determined separately for each trait, perhaps at different stages of ontogeny.

Finally, it has been suggested that asymmetry can alter during development due to compensational growth of the two trait sides (cf. Corruccini & Potter 1981; Emlen *et al.* 1993; Graham *et al.* 1993; Swaddle & Witter 1994). This implies some form of interaction or regulatory feedback between left and right sides. Initial asymmetries may be randomly determined, and as growth processes continue, we may expect asymmetries to decrease as compensational growth between the two sides will act to restore trait symmetry.

At present, there is a general lack of data from longitudinal investigations of trait development with respect to developmental stability. This kind of data provides valuable insights into the ontogeny of stability and, by helping to distinguish the relative pertinence of the six hypotheses described above, allows some interpretation of the mechanisms that underlie asymmetry production. Therefore, in this study we examined the ontogeny of asymmetries, that displayed the population characteristic of fluctuating asymmetry, in a trait under stabilizing selection (primary feathers) in European starlings, *Sturnus vulgaris*. Specifically, we examined the growth of left and right primary feathers

at regular intervals during moult and also compared asymmetries among feathers at the end of moult.

2. METHODS

We performed the experiment on ten wild-caught juvenile European starlings. As birds were caught in their first summer, they had already experienced natural photoperiodic cues to induce moult before their period of captivity. All birds were housed individually in $0.3 \times 0.3 \times 0.5$ m cages, in visual, but not acoustic, isolation and were provided with water and turkey starter crumbs *ad libitum*. Throughout the experiment, the birds were maintained on a photoperiod that simulated natural daylight and was reduced in 30-minute blocks, starting at 17.5 h of light and reducing to a $13:11$ h light:dark photoperiod at the end of moult. Every second day during moult we recorded length measurements of the seventh primary feather to 0.01 cm accuracy with Vernier callipers. All primary feathers were measured at the end of moult. The tips of primaries were always checked carefully to ensure that damaged feathers were not included in the analyses.

We identified three questions through which we could help to distinguish between the six non-mutually exclusive hypotheses for the ontogeny of fluctuating asymmetry (see table 1).

(*a*) Within individuals, are primary feather asymmetries consistently biased toward one side among feathers? Hypothesis (i) predicts a consistent bias, whereas hypotheses (ii), (iii), (iv), (v) and (vi) do not.

(*b*) Are primary feather length asymmetries consistently biased toward one side throughout feather growth within individuals? Hypotheses (i), (iii) and (v) predict that growth stages will be biased toward one side of the trait, whereas hypotheses (ii), (iv) and (vi) do not.

(*c*) Does asymmetry accumulate during the development of a trait? Hypotheses (i), (iii) and (iv) predict that asymmetry does accumulate as development continues; however, hypothesis (ii) suggests that relative asymmetry should decrease with growth, hypothesis (v) predicts that asymmetry should remain constant, and hypothesis (vi) predicts that both relative and absolute asymmetry will decrease with increasing stages of growth.

The repeatability of primary asymmetry measures was assessed by performing three repeated length measurements of left and right primaries 3 and 4 before the experiment began on all individuals. Primary asymmetry was highly repeatable (primary 3, $F_{9, 36} = 1592.55$, $p < 0.00001$; primary 4, $F_{9, 36} = 558.04$, $p < 0.0001$; see Swaddle *et al.* (1994) for details). It was assumed that measurement errors would be similar for all other feathers. All but two primaries in the juvenile experiment (primaries 2 and 8) displayed a characteristic half-normal distribution (Anderson–Darling normality test, $a^2 < 0.501$, $p > 0.1$; see Minitab Inc. (1994)) around a mean of zero (one-sampled *t*-test, $t < 1.58$, $p > 0.1$); hence primaries 2 and 8 were excluded from further analyses (Palmer & Strobeck 1986; Palmer 1994; Swaddle *et al.* 1994). Absolute asymmetry was defined as the unsigned difference between left and right sides ($|L-R|$); relative asymmetry was defined as absolute asymmetry divided by trait size ($(|L-R|)/0.5(L+R)$). Three juveniles died within the first two weeks of the experiment, reducing the experimental group to seven. These birds appeared to die of natural causes, as is often observed in juvenile birds. All analyses were performed using Minitab for Windows (Minitab Inc. 1994), employing two-tailed tests of probability throughout.

3. RESULTS

Signed primary feather length asymmetries, at the end of moult, were not consistently biased toward one side among feathers within individuals (Sign test, $N = 7$ primaries, $p > 0.12$ for all individuals). This is consistent with our previous studies of the development of asymmetries in adult starlings (J. P. Swaddle and M. S. Witter, unpublished data). This finding indicates that hypothesis (i) is not likely to be applicable in this case (see table 1).
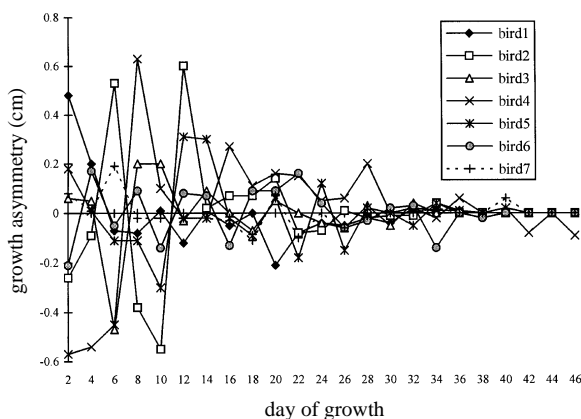

Figure 1. Signed growth increment (cm) over two consecutive days versus day of growth for primary seven. Each series represents one individual.
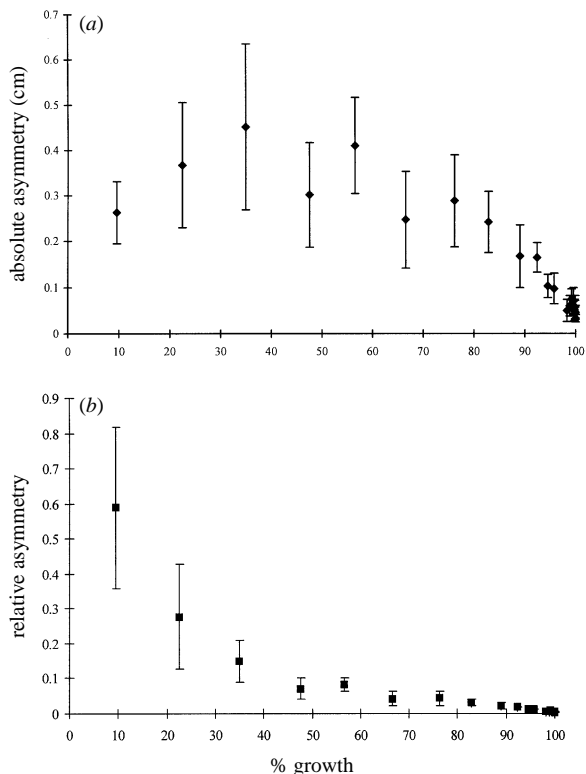

Figure 2. (*a*) Mean ($\pm$ s.e.) absolute asymmetry (cm) versus mean ($\pm$ s.e.) percentage growth, for primary seven. There is a significant negative relationship. (*b*) Mean ($\pm$ s.e.) relative asymmetry versus mean ($\pm$ s.e.) percentage growth, for primary seven. There is a significant negative relationship.

Growth increments of primary 7 were not consistently biased toward either the left or right sides within individuals (Sign test, $N = 23$ growth increments, $p > 0.50$ for all individuals; see figure 1). As the asymmetry of each growth increment was not sided, the data do not support hypotheses (i), (iii) and (v) (see table 1).

Absolute asymmetry decreases with increasing stages of growth of primary 7 (Spearman rank correlation, $r_s = -0.94$, $p < 0.0001$; figure 2a). Similarly, relative asymmetry decreases with growth stage ($r_s = -0.96$, $p < 0.0001$; figure 2b). As both indices of asymmetry decrease with increasing growth stages, these data tend to support hypothesis (vi) more strongly than the other hypotheses (see table 1). It should be noted that both relationships appear to be nonlinear, but the statistical properties of these data do not permit parametric regression analyses.

4. DISCUSSION

Our data indicate that primary feather asymmetries are not consistently biased toward one side in the same individual. The results also show that growth increments are not biased toward one side of the body within single feathers, i.e. the asymmetries constantly change in direction among growth stages (figure 1). Additionally, we have demonstrated that both absolute and relative asymmetry decrease as feathers reach their final growth stages (figure 2; cf. P. Barnard, unpublished data cited in Balmford *et al.* (1993); Swaddle & Witter (1994)). Overall, these findings tend to support hypothesis (vi) more strongly than the other hypotheses (see table 1), and hence indicate that the asymmetries we observed may arise through a mechanism of compensational growth between left and right wings. We should point out that most of this evidence relies on negative results (i.e. non-rejection of a null hypothesis) and hence does not provide the strongest possible support for hypothesis (vi), although the negative relations between asymmetry and stage of growth (figure 2) are convincing. Inspection of figure 1, which describes the direction and magnitude of the asymmetric growth increments recorded every other day, indicates that growth biased toward one side (i.e. left or right) on any particular day is often followed by a similar magnitude of growth biased toward the opposite side a few days later. The oscillations in growth increment asymmetry about the x-axis (i.e. zero asymmetry) appear to differ in amplitude and periodicity among individuals and hence are problematic to quantify statistically. Visual examination of this graph suggests that asymmetric growth on any given day may be followed by compensational growth biased in the opposite direction a few days later. Following inspection of figure 1, we performed an exploratory analysis that indicated that the signed growth increment at day x is negatively related to signed growth at day $x+4$, when all growth stages are pooled across feathers (signed growth at day $x+4 = 0.006 - 0.202$ (signed growth at day x); $F_{1,118} = 6.94$; $r^2 = 5.6\%$; $p = 0.010$). We should point

out that this analysis was *a posteriori*; we are not suggesting that starlings follow this growth trajectory as a general rule, although this does indicate that growth stages can be negatively related, hence possibly indicating some form of feedback or interaction between left and right sides during development.

However, we should point out that there is evidence to indicate that fluctuating asymmetry does not always follow similar developmental patterns in traits in other species. Chippindale and Palmer (1993) observed that limb-segment asymmetries of crabs, *Hemigrapsus nudus*, generally did not change in magnitude or directionality between successive moults on the same individual. These data suggest that both the size and sign of these left–right asymmetries are determined at an early stage of development and persist through subsequent moult and regrowth, and hence may support hypothesis (v), the persistent asymmetry hypothesis. It is possible that the asymmetries Chippindale and Palmer measured were genetically determined, although selection experiments with *Drosophila* have repeatedly failed to indicate a strong genetic component to the size and direction of developmental asymmetries (Maynard Smith & Sondhi 1960; Coyne 1987; Tuinstra *et al.* 1990). Chippindale and Palmer suggest that the asymmetries in their crabs' limbs were not corrected for between moults because the asymmetries are small ($< 1\%$ of trait size) and there may be some threshold level below which asymmetries remain uncorrected. This may help to explain the discrepancy between their data and the findings reported in this study. Asymmetries of starling primary feathers are functionally costly in terms of flight performance (Balmford *et al.* 1993; Swaddle *et al.* 1996), whereas small asymmetries of crab limbs may have little functional consequence; hence primary feather asymmetries are corrected and crab limb asymmetries persist. This suggests that the ontogeny of developmental stability may differ among traits of varying functional importance, which can be tested directly through comparison of different traits (e.g. ornamental traits versus biomechanically functional traits) on the same individuals.

Møller (1996) has also shown a consistent between-moult side-bias in the development of tail feather asymmetries in barn swallows, *Hirundo rustica*. His data also indicated that asymmetries in growth bars (developmental units) were positively related to final feather asymmetries, and that there was a constant (left–right) asymmetry bias in growth bar length (i.e. one side was consistently larger than the other). These data provide general support for a magnification of persistent asymmetries over development, although there was no relation between tail asymmetry and number of developmental units in this study. Therefore, these data provide general support for both hypotheses (iii) and (v). Again, the difference in functional importance of an ornamental tail trait versus a primary flight feather may help to explain the discrepancy between Møller's (1996) findings and those reported here.

If some form of regulatory feedback does occur during the development of bilateral asymmetry, it may occur through communication via neuronal or cir-

culatory systems, or via hormonal regulation (Emlen *et al.* 1993). Young *et al.* (1994) have demonstrated that neural inhibition may be a fundamental aspect of determining claw antisymmetry in snapping shrimps, *Alpheus heterochelis*. It may also be relevant that Graham *et al.* (1993) have proposed a theoretical model of non-linear feedback that regulates the production of asymmetry through the relative action of diffusible morphogens. We envisage that feedback may occur in a number of different ways. First, there could be direct communication between the left and right elements of a trait. Second, information could be sent indirectly to and from the left and right sides via a central controller (e.g. the central nervous system). Finally, there could both be direct and indirect feedback between the two sides of the trait. Only through detailed neurological and biochemical studies will it be possible to investigate which of these speculative mechanisms operate during the production of fluctuating asymmetry in bilateral traits. It is likely that the relative activity of these feedback mechanisms will alter during ontogeny and, also, there may be phases of development that are particularly sensitive to disruptions of developmental stability (cf. Møller 1996). Feedback mechanisms are also likely to differ among traits, and the distance over which the communication occurs may be an important factor influencing these mechanisms.

We encourage further and more detailed studies of the mechanisms that give rise to developmental instability, as this is a crucial element in interpreting the evolutionary importance of fluctuating asymmetry. Without knowing how asymmetries are produced or regulated, it is difficult to make conclusive statements relating to exactly what asymmetries reveal about the organism or population. It has often been claimed that fluctuating asymmetry reveals information concerning the genetic make-up of organisms (reviews in Palmer & Strobeck (1986); Parsons (1990); Møller & Pomiankowski (1993); Watson & Thornhill (1994)) and yet the developmental steps from genotype to phenotype are far from clear. These kinds of studies will provide valuable insights into the current asymmetry–signalling debate (see Palmer 1996). The issue of whether a negative relation between trait length and asymmetry has arisen due to sexual or natural selection has been the topic of recent debate (see Møller 1992, 1993; Balmford *et al.* 1993; Evans & Hatchwell 1993). Additionally, it has been proposed that asymmetry may change during the growth of traits (Balmford *et al.* 1993; Swaddle & Witter 1994). Studying the ontogeny of developmental asymmetries will help to shed light on the relative mechanical constraints acting on trait design and help to assess whether patterns of asymmetry are determined primarily by sexual or natural selection forces.

In summary, the data we present here from starling primary feathers support the hypothesis that bilateral asymmetry is regulated by some form of feedback and compensatory growth during development. Asymmetries are large at the beginning of development, but as the trait continues to grow both relative and absolute asymmetry decrease, seemingly through episodes of compensational growth of left and right primary feathers. However, the relevance of this hypothesis to the ontogeny of asymmetry and developmental stability in other traits and other species remains unclear. We predict that the functional importance of traits will affect asymmetry production and may influence the mechanisms by which the asymmetries develop. In traits where asymmetry imposes little cost, there will be weak selection for feedback, and compensatory mechanisms and asymmetries may either persist or accumulate over developmental stages.

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