

The Ontogeny of Fluctuating Asymmetry

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ABSTRACT: We tested seven hypotheses regarding the mechanisms by which fluctuating asymmetry (FA) originates. We did this by analyzing data on four bilateral characters measured repeatedly during the development of individual domestic fowl. Immediately post-hatching, there was substantial directional asymmetry, which rapidly decreased. We detected FA at significant levels in all characters in the majority of our measurements over the remainder of development. We also examined the effects of known environmental stressors (food and density stress) on levels of FA. At the levels we examined, changes in these stressors did not alter the degree of asymmetry we found in fowl. Time series of asymmetry for individuals did not exhibit regular oscillations, as much of the relevant literature predicts. Asymmetry levels reflected the combined effects of developmental noise, which was random in degree and direction, and feedback processes, which decreased asymmetry by altering growth rates on both sides of the body. Our findings best fit the predictions of the residual asymmetry and compensatory growth hypotheses, which suggest that levels of asymmetry reflect only recent growth history.

Keywords: fluctuating asymmetry, bioindicator, ontogeny, developmental instability, stress.

Developmental instability (DI) analysis can be used to make inferences about the general health of populations. It is based on the assumption that environmentally or genetically induced deviations from the ideal phenotype provide information about the precision of development, with lower precision reflecting the disruptive effects of environmental stressors, poor genetic quality, or both (reviewed by Leary and Allendorf [1989]). The most commonly used index of developmental instability is fluctu-

ating asymmetry (FA), the variance in right minus left ($R_i - L_i$) values of bilaterally symmetrical structures (Palmer and Strobeck 1992). Fluctuating asymmetry is characterized by a normal distribution of $R_i - L_i$ values about a mean of 0.

In addition to FA, two other types of bilateral asymmetry exist: directional asymmetry and antisymmetry (Van Valen 1962). Directional asymmetry occurs where there is a consistent bias for one side to become larger than the other side (e.g., the mammalian heart shows directional asymmetry, with the left side larger than the right; Van Valen 1962). Antisymmetry is characterized by a bimodal distribution of $R_i - L_i$ with a mean of 0 (e.g., the signaling claws of the male fiddler crab; Graham et al. 1993a). There is disagreement within the literature regarding whether the different types of asymmetry represent different products of the same underlying processes or result from fundamentally different processes. Palmer and Strobeck (1992) argued that only FA is useful for studies of developmental instability. They suggested that because directional asymmetry and antisymmetry do not have an ideal state that is definable a priori, it is impossible to separate asymmetry caused by DI from that which has a genetic basis (Palmer and Strobeck 1992). However, antisymmetry was observed in Australian sheep blowflies *Lucilia cuprina* during their evolution to pesticide resistance (McKenzie and Clarke 1988), and Graham et al. (1993b) induced significant fluctuating asymmetry and directional asymmetry in *Drosophila melanogaster* exposed to high concentrations of benzene. The debate over which types of asymmetry can reflect DI is likely to remain unresolved until we understand the developmental origins of asymmetry of various types.

Swaddle and Witter (1997) compiled a list of six hypotheses that attempt to explain how asymmetry arises during growth and development: the directional external cues hypothesis, the "coin-toss" hypothesis, the magnification of asymmetry hypothesis, the accumulation of accidents hypothesis, the persistent asymmetry hypothesis, and the compensatory growth hypothesis. We developed predictions from these hypotheses and added a seventh hypothesis, which we call the residual asymmetry hypothesis. We then designed experiments to distinguish among

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these predictions. A summary of the predictions generated by each of the seven hypotheses is presented in table 1.

The directional external cues hypothesis suggests that side-biased environmental influences can induce asymmetrical growth and development within individuals (Grüneberg 1935; Hallgrímsson 1993, 1998, 1999). These influences may be external, such as light or currents affecting sessile plants or animals, or internal, such as the influences of "handedness" biases in usage on the development of bones and muscles (Hallgrímsson 1998, 1999). Directional external cues could lead to FA or antisymmetry at the population level, depending on the degree to which the strength of the biased cue varies among individuals. Regardless of the source of the bias, this hypothesis predicts that time series measured within individuals should constantly diverge from symmetry at rates and in directions that depend on the strength and direction of cue bias within individuals (fig. 1A).

The coin-toss hypothesis suggests that structures grow by the accumulation of independent morphological subunits. Asymmetry between two traits is the cumulative result of differences in sizes between corresponding morphological units, which are determined by chance (Hallgrímsson 1993, 1998, 1999). This hypothesis therefore predicts that, on average, relative levels of asymmetry within individuals should vary early, then decrease throughout most of ontogeny (fig. 1B). This is dictated by elementary sampling theory (Lande 1977; Soulé 1982), which states that the size and variance in the size of a structure composed of independent subunits will increase in proportion to the number of subunits n , but the coefficient of variation (CV) will increase in proportion to the square root of n . Because asymmetry is the difference between two such composite structures, when the CVs of both sides decrease, the expected relative difference between them will decrease as well.

The magnification of asymmetry hypothesis states that small variations in the initial growth conditions of a structure can be magnified by subsequent morphogenesis, leading to larger final asymmetry (Emlen et al. 1993; Hallgrímsson 1993, 1998, 1999). Random differences in initial rates of cell division between corresponding bilateral structures would cause their initial rates of growth to differ. Because structures initially consist of few cells, small differences in rates of cell division should lead to proportionally large differences in structure size. These proportionally large but absolutely small differences should be magnified in absolute size by subsequent growth, which should occur at increasingly similar rates between sides as their size increases. The increasing similarity in growth rates between sides with increasing size should occur because as the number of sampling units (cells) increases, the mean rate of division, and thus growth, should con-

verge between sides. By this mechanism, initial proportional differences in size can be fixed early in development (fig. 1C) and subsequently remain constant while absolute asymmetry is magnified.

The accumulation of accidents hypothesis suggests that the developmental program does not target perfect symmetry but aims for a range of $R_i - L_i$ values about perfect symmetry (Hallgrímsson 1993, 1998, 1999). As long as developmental noise does not cause asymmetry to depart from this range, asymmetry will follow a random walk through time. In consequence, unless the target range of $R_i - L_i$ values is exceeded, the population variance in $R_i - L_i$ values will increase as ontogeny progresses (fig. 1D). If the target range is exceeded, relative asymmetry levels should increase, on average, through ontogeny until the boundaries are reached and then should remain relatively constant (fig. 1E).

The persistent asymmetry hypothesis suggests that departures from symmetry can be determined genetically or through environmental effects early in ontogeny and that the sign and magnitude of asymmetry should persist over time (fig. 1E; Chippendale and Palmer 1993). Because this hypothesis is entirely phenomenological, its predictions cannot be distinguished under all circumstances from those of the directional external cues and magnification of asymmetry hypotheses.

The compensatory growth hypothesis suggests that because large random differences in the size of bilateral structures are not the norm, feedback mechanisms must exist (Emlen et al. 1993). There are two ways that these mechanisms might operate: first, negative feedback among cells might act to suppress biosynthesis on the side that is too large. Second, there may be positive feedback between right and left structures (Emlen et al. 1993). Positive feedback between right and left sides via the nervous or circulatory systems would maintain symmetry by promoting catch-up growth on the lagging side (Emlen et al. 1993). This, in turn, could lead to right-left oscillations in asymmetry, the magnitude of which would be a function of the individual's rate of growth, the communication time gap, and the strength of the catch-up signal (fig. 1G; Waddington 1957; Emlen et al. 1993).

Our seventh hypothesis, the residual asymmetry hypothesis, suggests that there are simple compensatory mechanisms that act to counter the effects of developmental noise and that they respond continuously to the morphology of the individual with relatively short time lags and thus produce weak or no long-term temporal patterns (fig. 1H). Under this hypothesis, the level of asymmetry in each animal is the residual result of developmental noise minus correction, and the level of FA in the population reflects only recent levels of developmental noise (Van Valen 1962). This hypothesis is a subset of the

Table 1: Predictions for within-individual patterns of asymmetry based on seven hypotheses

Questions	Directional external cues	Coin toss	Magnification of asymmetry	Accumulation of accidents	Persistent asymmetry	Compensatory growth	Residual asymmetry	Data from four traits
Do individual asymmetry time series constantly diverge from symmetry?	Yes	No	Yes	No	Yes	No	No	No
Does size-relative asymmetry decline constantly throughout ontogeny?	No	Yes	No	No	No	No	No	No
Are changes in asymmetry between measurements determined by chance?	No	Yes	No	Yes	No	No	No	No
Do future changes in asymmetry occur in directions to reduce current asymmetry?	No	No	No	No	No	Yes	Yes	Yes
Do asymmetry time series exhibit regular oscillations of a common period?	No	No	No	No	No	Yes (but not essential)	No	No

Note: Results in bold are supported by two experiments in which repeated measurements of right-left asymmetry were analyzed for four traits in individual domestic fowl: length of the tarsometatarsus, width of the tarsometatarsus at the spur, width of the tarsometatarsus at the upper joint, and the distance from the naris to the commissure of the mandibles.

compensatory growth hypothesis in that it excludes the possibility of regular oscillations in asymmetry between right and left sides.

We have summarized the features of the predictions of the seven hypotheses regarding the origin of asymmetry that make it possible to distinguish among them in table 1. It is clear that it is possible to determine which hypothesis best characterizes the development of asymmetry in a species using data on patterns of change in asymmetry measured in individuals over time. It is important to discriminate among these hypotheses because the implications of differences in levels of asymmetry among populations depend strongly on which of the seven hypotheses are correct for the species studied. The directional external cues hypothesis, the magnification of asymmetry hypothesis, and the persistent asymmetry hypothesis each suggest that present asymmetry levels can reflect short-term incidents that occurred early in development. Differences in FA levels might thus indicate that populations have experienced different levels of environmental stress for short periods at some point in the past. Under the accumulation of accidents hypothesis, either a short period of strong stress or a prolonged period of less intense stress would increase the variance of the locations of individuals along their random walks and thus increase final population FA levels. Under the compensatory growth and residual asymmetry hypotheses, differences in asymmetry at any time should reflect recent differences in stress levels, while under the coin-toss hypothesis, FA should largely reflect recent stress levels and should decrease with increasing body size at any stress level.

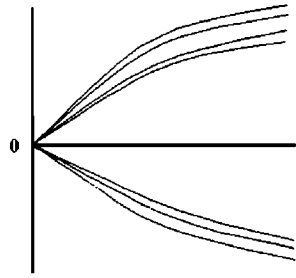
Understanding the implications for population histories of differences in asymmetry levels is important because monitoring programs that compare developmental insta-

bility among populations or over time may provide a type of early warning system that would alert biologists to the presence of a stressing agent and impending population declines (e.g., Leary and Allendorf 1989; Parsons 1990; Clarke 1995; Alford et al. 1999; Lens et al. 2002). This, in turn, could allow identification, and possibly alleviation, of the causes of stress before they adversely affect population numbers (Leary and Allendorf 1989; Clarke 1995; Alford et al. 1999; Lens et al. 2002).

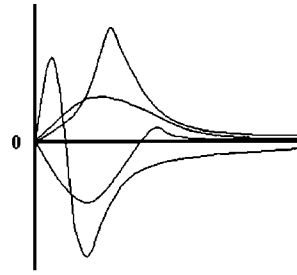
One approach to understanding the mechanisms that determine levels of asymmetry is to take repeated measurements of individuals' right-left asymmetry throughout growth and development and compare the observed patterns to those predicted by models. A few studies have attempted this (Chippendale and Palmer 1993; Swaddle and Witter 1994, 1997; Møller 1996; Teather 1996; Collin 1997; Aparicio 1998; Tomkins 1999). However, none of these studies has rigorously tested all of the hypotheses regarding the ontogenetic origin of FA. Doing so was the impetus for our study.

We aimed to determine which of the hypotheses concerning the ontogeny of asymmetry best fits data on individual growth histories and to investigate the effects of two potential environmental stressors (low food levels and increased density) on the ontogeny of asymmetry. We addressed these aims by analyzing repeated measurements of bilateral structures taken on individual domestic fowl (*Gallus gallus domesticus*) exposed to hunger-stressed, density-stressed, and benign conditions during their ontogeny. To examine the effects of stressors, we compared levels of expressed asymmetry between fowl in low- and high-stress treatments. We discriminated among the seven hypotheses regarding the ontogeny of asymmetry by comparing observed temporal patterns of asymmetry to the

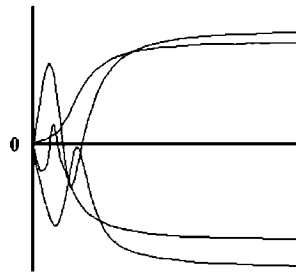
Relative asymmetry



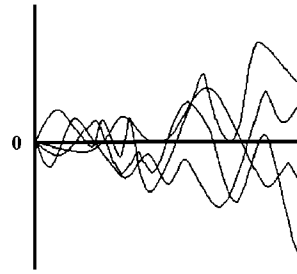
A. Directional external cues



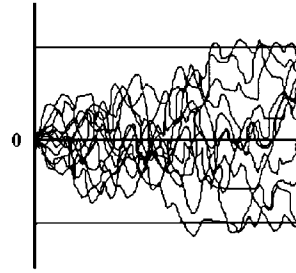
B. 'Coin-toss'



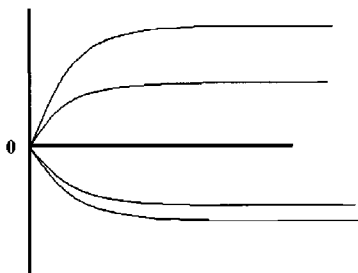
C. Magnification of asymmetry



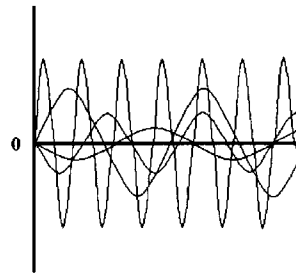
D. Accumulation of accidents



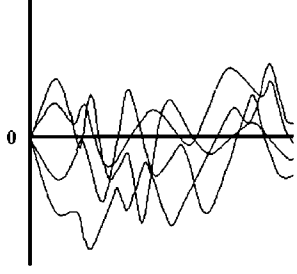
E. Accumulation of accidents to boundaries



F. Persistent asymmetry



G. Compensatory growth



H. Residual asymmetry

Time

patterns we predicted from the hypotheses (fig. 1; table 1). We also developed a simulation model of bilateral growth based on the hypothesis that best fit the data, estimated its parameters from the data, and compared the temporal patterns of asymmetry of real and simulated fowl to determine whether the simulation model adequately reproduced the patterns observed in animals.

Methods

Experiments

We performed two separate experiments, and data from both were used to discriminate among the seven hypotheses regarding the ontogeny of asymmetry. The first experiment also investigated the effects of hunger stress, while the second provided finer temporal resolution and examined the effects of rearing density. For both experiments, we reared Loman Brown pullets (*Gallus gallus domesticus*), a fast-growing breed of chickens that reach adult size in approximately 42 d. The experiments were carried out in a controlled-temperature room. Pullets were reared in cardboard pens measuring 41 cm in length \times 38.4 cm in height \times 41 cm in width, which visually isolated the individuals within them from those in other pens. Pens were arranged on shelving in four separate blocks, each consisting of five columns and two rows.

In both experiments, the room temperature was held at 29°C for the first week. Following standard procedures for raising fowl, it was subsequently decreased gradually each day, reaching 21°C at the end of the third week, after which this temperature was maintained for the remainder of the study. Temperatures up to 6°C above the ambient room temperature were provided using overhead 25-W light bulbs suspended over the corners of pens, so that chicks could position themselves near warmth as needed.

The first experiment was designed to compare the ontogeny of asymmetry in food-stressed birds to that in control birds. Birds were fed daily, and water was supplied to all birds ad lib. Twenty-four pens were assigned to the control treatment and 16 pens were assigned to the food-stress treatment. Pens were arranged in blocks of five so that the control treatment was replicated at three random locations within each block, and the food-stress treatment was replicated at two random locations within each block. Two newly hatched pullets were randomly allocated to each pen at the start of the experiment. Control birds were fed commercial food (Riverina Chick Starter Crumble) ad lib.,

with the difference between food supplied and that left over used to determine their intake. Food-stressed birds were allowed 80% of the body weight specific food intake of control birds of the same age. The quantity of food to be supplied to the birds in each pen in the food-stressed treatment was calculated every second day by multiplying the mean food consumption (g) per gram of body mass of the control group for the preceding day by 0.8 times the mass (g) of the birds in each food-stress pen.

The second experiment consisted of two components: the first was a more detailed investigation of the ontogeny of asymmetry, and the second was an examination of the effects of a density stress on asymmetry. Results of the first experiment suggested that measuring animals more frequently might allow us to determine more accurately how quickly growth changes to compensate for changes in asymmetry during ontogeny. We also wanted to examine the effects of density stress on the ontogeny of asymmetry. Møller et al. (1995) found that chickens reared at densities of 24 and 28 per square meter had higher levels of asymmetry than individuals reared at 20 individuals per square meter. We exceeded this density range by rearing pullets in 20 pens containing two pullets each (12 birds per square meter) and in 20 pens containing five pullets each (30 birds per square meter). Each treatment was replicated five times in each experimental block, and treatments were allocated to pens so that they alternated within each column and row. Pullets were assigned to each pen at random, and food was supplied to all pens ad lib.

Measurement and Analysis of Asymmetry

It has been suggested that measurement of several traits rather than of one trait and the use of composite FA analyses (CFAs) should increase the power to detect differences in FA between stressed and unstressed populations (Leung et al. 2000). We therefore measured four bilateral traits: length of the tarsometatarsus, width of the tarsometatarsus at the spur, width of the tarsometatarsus at the upper joint, and the distance from the naris to the commissure of the mandibles. The first three of these traits were also measured by Møller et al. (1995). In both experiments, one individual in each pen was chosen at random for the first measurement; the same individual was subsequently re-measured at each time. During the first experiment, measurements were taken every 48 h for 34 d. In the second experiment, measurements were taken on low-density

Figure 1: Predictions of individual asymmetry time series according to the directional external cues hypothesis (A); coin-toss hypothesis (B); magnification of asymmetry hypothesis (C); accumulation of accidents hypothesis (D, E); persistent asymmetry hypothesis (F); compensatory growth hypothesis (G); and residual asymmetry hypothesis (H).

birds every 12 h for 10 d, followed by a single set of measurements taken on both high- and low-density birds on days 38 and 39 of the experiment. Measurements were always made by the same individual using Mitutoyo electronic calipers accurate to 0.01 mm.

Measurements needed to be highly accurate because small differences in asymmetry can be important (Palmer and Strobeck 1986; Palmer 2000). Therefore, we repeated each measurement on each individual three times during each measurement session, and means of the three replicate measurements were used in most analyses. To avoid measurement bias, we used the “single-blind” method detailed by Alford et al. (1999). Three sets of measurements of all characters on both sides of each individual were taken, and the digital readout of the calipers was covered to ensure that the values could not be observed while measuring. Data were fed directly into a spreadsheet on a recording computer by pressing the data output button on the calipers after each measurement.

Leung et al. (2000) reviewed and tested the efficacy of six indices that can be used to compare composite levels of FA between populations. They exposed difficulties (i.e., inflated Type I error rates, decreased power) in four of those indices and suggested that the decision regarding which of the remaining two would be most powerful should be made depending on levels of kurtosis within the particular distributions of interest.

Both of the indices recommended by Leung et al. (2000) facilitate comparison of composite FA between populations by standardizing differences in FA between traits of differing sizes and by standardizing developmental instability between traits. In creating composite indices of FA, it is important to scale out inherent differences in developmental instability between traits even after scaling for differences in trait size. This is because baseline levels of asymmetry can differ among traits (Leung and Forbes 1997). Small but significant increases in levels of FA in traits with low baseline levels could be obscured by traits that may not respond greatly to stress but merely have normally high levels of FA.

Although scaling out differences in developmental instability between traits is useful when one does not know how traits might differ in DI, if traits are actually similar in baseline levels of DI, scaling for differences in DI will reduce the power of statistical tests to detect true differences in magnitudes of fluctuating asymmetry between populations. Therefore, to compare the magnitude of FA between treatments, we calculated one composite FA score that does not control for differences in developmental instability and two composite FA scores, as suggested by Leung et al. (2000), that do control for differences and compared them using three simple *t*-tests with Bonferroni adjustment.

The first score, CFA 1, does not scale out differences in developmental instability between traits but does scale levels of asymmetry relative to trait size such that the scaled value of asymmetry for individual *i*, trait *j*, (SA1_{*ij*}) is

$$SA1_{ij} = \frac{|A_{ij}|}{\frac{1}{2}(R_{ij} + L_{ij})}$$

That is, the standardized asymmetry value is the absolute asymmetry value within individual *i*, trait *j*, divided by the average length of trait *j* of individual *i*.

The second composite FA score (CFA 2 in Leung et al. 2000) was calculated by summing standardized asymmetry values across all traits for each individual such that the scaled asymmetry value for individual *i*, trait *j*, (SA2_{*ij*}) is

$$SA2_{ij} = \frac{|A_{ij}|}{\frac{1}{n} \sum_{i=1}^n |A_{ij}|}$$

That is, the standardized asymmetry value is the absolute asymmetry value within individual *i*, trait *j*, divided by the average over all individuals of the absolute asymmetry of trait *j*. The third score (CFA 3 in Leung et al. 2000) was calculated by ranking unsigned asymmetry values within traits and then summing ranked values across traits for each individual.

We used SAS 6.12 to carry out a nested ANOVA on all of the data and to calculate FA values and estimates of measurement error using mean squares as suggested by Palmer and Strobeck (1986, 2003). The ANOVA tests for directional asymmetry by determining whether significant differences exist between the sizes of each trait on the left and right sides of animals. It also determines whether FA is greater than measurement error by calculating the ratio between the variance associated with the side by individual interaction and the variance among replicate measurements within individuals. Because our design was balanced, the sums of squares we used to test for the significance of FA and directional asymmetry are additive. It is therefore possible to detect both significant FA and significant directional asymmetry in the same population. We initially performed these analyses on untransformed character sizes and found that the estimates of FA we obtained were significantly positively correlated with the average body size of birds at each time in each treatment for three of the four characters in each experiment. We therefore transformed character size before these analyses by converting all replicate right and left measurements to their natural logarithms (Palmer and Strobeck 2003). We used the mean squares from these analyses to calculate population estimates of FA:

$$\text{FA10b} = 0.798 \sqrt{2 \left(\frac{\text{MS}_{s_j} - \text{MS}_m}{M} \right)},$$

where MS_{s_j} is the mean square for the side \times individual interaction in an ANOVA on log-transformed data to detect FA, MS_m is the error mean square for that ANOVA, and M is the number of replicate measurements on each individual (Palmer and Strobeck 2003) for each treatment in each experiment at each time of measurement. We also used the MS to calculate measurement error ($\text{ME2} = \text{standard deviation of replicate measurements within individuals} = \text{square root of } \text{MS}_m$; Palmer and Strobeck 1986, 2003) for each treatment in each experiment at each time of measurement. We examined the relationships of these estimates to body size and day to determine how population levels of FA and measurement error changed through time for each character.

We searched for the presence of periodic oscillations within individual time series using spectral decompositions. Time series that contain regular oscillations will produce spectral decompositions with significant contributions from waves with periods that reflect the inherent periodicities in the data, regardless of phase. Comparing the decompositions of many time series allows determination of whether they share common periodic structure. We carried out spectral decompositions on the time series of each trait for each individual; these were tested for significant departures from white noise using Bartlett's Kolmogorov-Smirnov statistic (SAS Institute 1989).

Even if there are no periodic oscillations within a time series, it is possible that succeeding values reflect a combination of random variation and feedback processes. To determine whether feedback processes were operating, we investigated autocorrelation functions (ACFs) between the magnitude of asymmetry at time t (A_t) and future values of changes in asymmetry (ΔA). A relationship between A_t and the change in asymmetry from time t to $t + \tau$ ($\Delta A_{t,t+\tau}$) where τ denotes the interval between measurements would suggest that a short-term feedback process was operating. A relationship between A_t and ΔA at higher lags could be caused directly by long-term effects of A_t on ΔA at higher lags (i.e., the magnitude of asymmetry at time t dictates relative growth rates of right and left structures beyond $t + \tau$) or indirectly because of autocorrelations between values of A_t . To discriminate between these possibilities, we controlled for asymmetry at $t + \tau$ ($A_{t+\tau}$) by examining partial autocorrelation functions (partial ACFs) between A_t and the change in asymmetry from $t + \tau$ to $t + 2\tau$ ($\Delta A_{t+\tau,t+2\tau}$). In each analysis, we calculated correlations separately for the time series for each individual and tested the null hypothesis that the mean over all individuals of the correlation was 0 by using t -tests.

We analyzed the time series data from both experiments using this approach.

Although the second experiment provided greater temporal resolution than the first, it was possible that this was greater resolution on a process different from that detected in the first experiment. To determine whether the processes operating in the second experiment were similar to those in the first experiment, we arranged the data from the second experiment into 48-h increments and compared the correlation between A_t and $\Delta A_{0,48}$ for each trait in the second experiment with that for the same trait in the first experiment.

There are at least two ways that organisms could correct for asymmetry between right and left sides (Emlen et al. 1993). Positive feedback between sides would restore symmetry by initiating catch-up growth on the lagging side. Conversely, negative feedback would act to suppress biosynthesis on the larger side, allowing the lagging side to catch up. We performed a series of analyses to determine which of these mechanisms was operating in domestic fowl by investigating the relationship between A_t and the corresponding residuals (i.e., same time and animal) from linear regressions of growth versus size for each character on each side of each animal. Because A_t is $R_t - L_t$, the signed differences between sizes of the structures on the right and left sides of the body at time t , significant negative correlations between A_t and the residual of right-side growth and significant positive correlations between A_t and the residual of left-side growth both indicate that compensatory growth is occurring. We determined whether overall correlations were significant using t -tests of the hypothesis that the mean correlation taken over all individuals in each experiment was equal to 0.

Comparisons of Real and Simulated Time Series

We used the results of the correlation and regression analyses to estimate the parameters of first-order autoregressive models (AR[1]) that predict the changes of asymmetry through time in animals following the residual asymmetry hypothesis. The models predicted asymmetry values for each of a series of time steps according to the equation

$$A_t = A_{t-\tau} + (A_{t-\tau} \times \beta) + \varepsilon_{ijt},$$

$$A_0 = \alpha,$$

where α is the overall mean asymmetry of a structure and would normally be set to 0 but was set to the measured overall mean asymmetry of each trait because the chickens exhibited a very small degree of directional asymmetry in some characters; β was estimated from the slope of the best-fit regression line of $\Delta A_{t,t+\tau}$ on A_t ; and ε_{ijt} represents

normally distributed random variation, simulating developmental noise. For our simulations, the magnitude of the standard deviation of this parameter was set to be equal to the standard deviation of the distribution of A_i taken over the whole experiment, using the equation

$$\varepsilon_{ijt} = x \times s.$$

The term s in this equation describes the standard deviation of the trait being simulated in the population whose parameters are to be compared to those estimated from the simulation model. The x represents a normally distributed random number with mean 0, standard deviation 1, generated using the equation

$$x = \sqrt{-2 \ln q_1} \times \sin(2\pi \times q_2),$$

where q_1 and q_2 are uniformly distributed random numbers on the interval 0, 1. We used each model to generate time series of lengths matching the time series measured in experiments 1 and 2 for 5,000 simulated birds. We calculated correlations for each time series and used box plots (Tukey 1977) to graphically compare the distributions of these correlations to the distributions of correlations from the original birds to determine whether the models behaved similarly to the real animals.

All procedures within this study complied with the James Cook University guidelines for the housing and care of laboratory animals and received ethics approval from the James Cook University ethics committee (approval A561).

Results

The Magnitude of FA and Measurement Error

Data from the first two measuring sessions of both experiments were excluded from analyses owing to evidence of strong directional asymmetry, which decreased rapidly in the first few days posthatching (fig. 2). We examined the distributions of $(R_i - L_i)$ for all traits in each treatment of each experiment, scaling out effects of body size by log-transforming the measurements (Palmer and Strobeck 2003). Histograms of each distribution appeared to closely match normal curves, and comparing kurtosis statistics to the table of critical values in Palmer and Strobeck (2003) showed that none was significantly platykurtic. This indicates that departures from symmetry were due to FA and measurement error with little or no contribution from antisymmetry. Results of nested ANOVAs applied to the log-transformed data for each day showed that the magnitude of FA was significantly greater than the magnitude of measurement error much more frequently than would

occur by chance in most traits of both experiments (83 of 128 combinations of character, treatment, and time of measurement in the first experiment vs. a maximum upper 95% binomial confidence limit of 10 expected for Type I error; 57 of 76 in the second experiment vs. an upper 95% expectation of 7 with no apparent pattern to combinations of time and character in which FA did not exceed measurement error; fig. 3). Figure 3 also indicates that measurement error (ME) tended to decline over time in both experiments (significant decreases with time in three characters in experiment 1 and two in experiment 2), while FA in three of the four characters remained nearly constant over the longer periods of experiment 1 and increased slightly with time in three of the four characters over the shorter, more intensive periods of experiment 2. Analyses of data from the first 8 d of experiment 1 produced patterns similar to those shown for experiment 2, indicating that in most characters, FA levels increased early in growth and then remained constant, while ME decreased throughout growth.

The nested ANOVAs also confirmed that the means of the distributions of $R_i - L_i$ deviated from 0 for most traits in both experiments at most measurement times (111 of 128 combinations of character and day of measurement in the first experiment; 61 of 76 in the second experiment). The only exception was the tarsometatarsus upper width in the second experiment, where directional asymmetry was present in only four sets of measurements. Our orthogonal sums of squares meant that directional asymmetry was extracted independently from FA and measurement error in our ANOVAs, and it was always small relative to the levels of FA expressed (fig. 2); we therefore ignored its existence in later analyses (Palmer and Strobeck 2003).

Correlations of unsigned asymmetry values among pairs of characters calculated across all individuals and measurement dates within each of the two experiments revealed that FA levels were not highly correlated among the four characters. Coefficients of determination ranged from a minimum of <0.005 to a maximum of 0.038. This indicates that the composite asymmetry indices we used to examine the effects of stress on FA were calculated on characters whose asymmetry levels varied almost completely independently within individuals.

The Effects of Stress on FA

We examined the effects of stress in both experiments by comparing means of composite asymmetry indices on the final measurement day between treatment groups using Bonferroni-adjusted t -tests. In the first experiment, the mean mass on day 34 of the hunger-stressed group

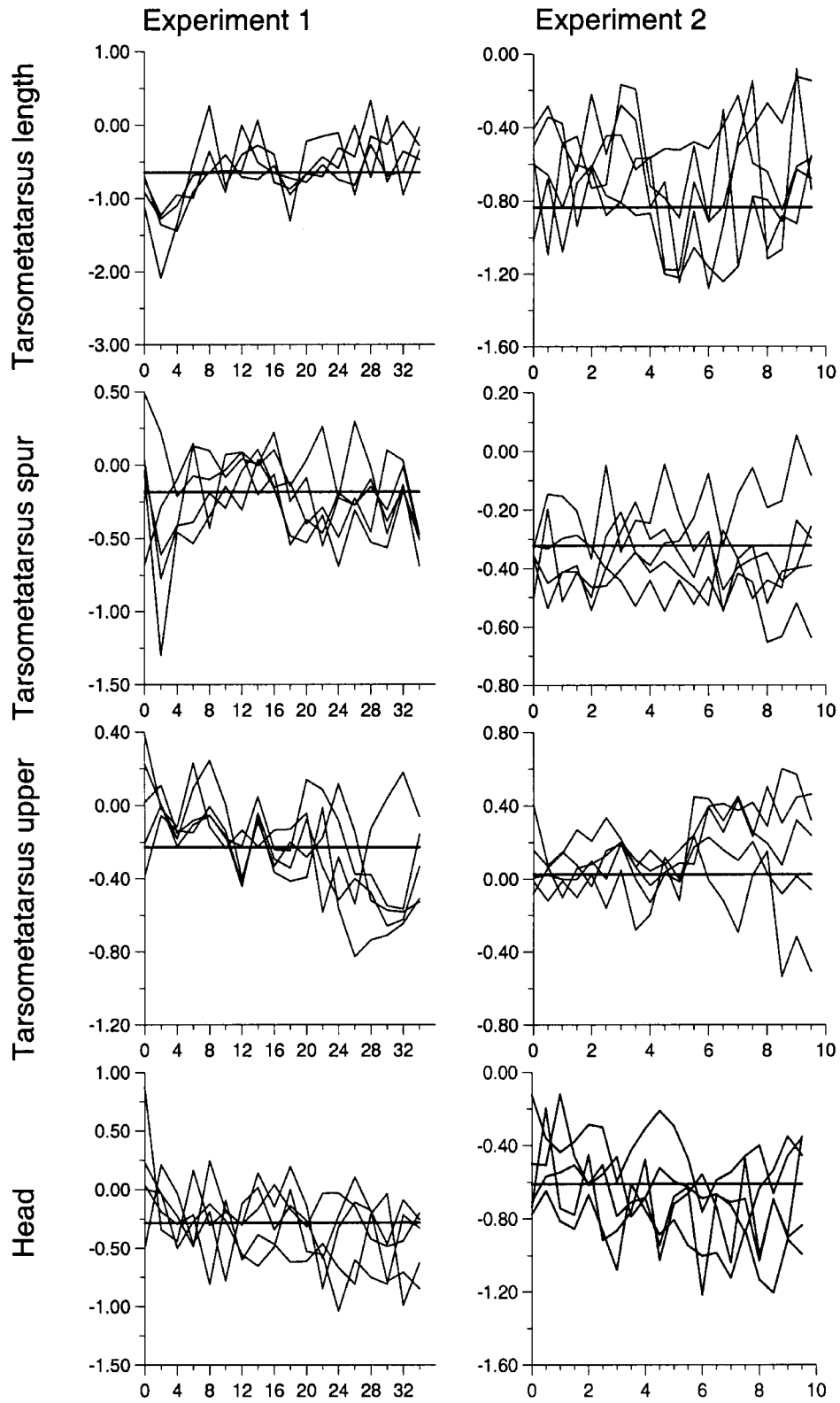


Figure 2: Selected examples of individuals' asymmetry time series. Flat line indicates mean level of asymmetry over all measurements for entire group. Y-axis shows $R_i - L_i$ (mm).

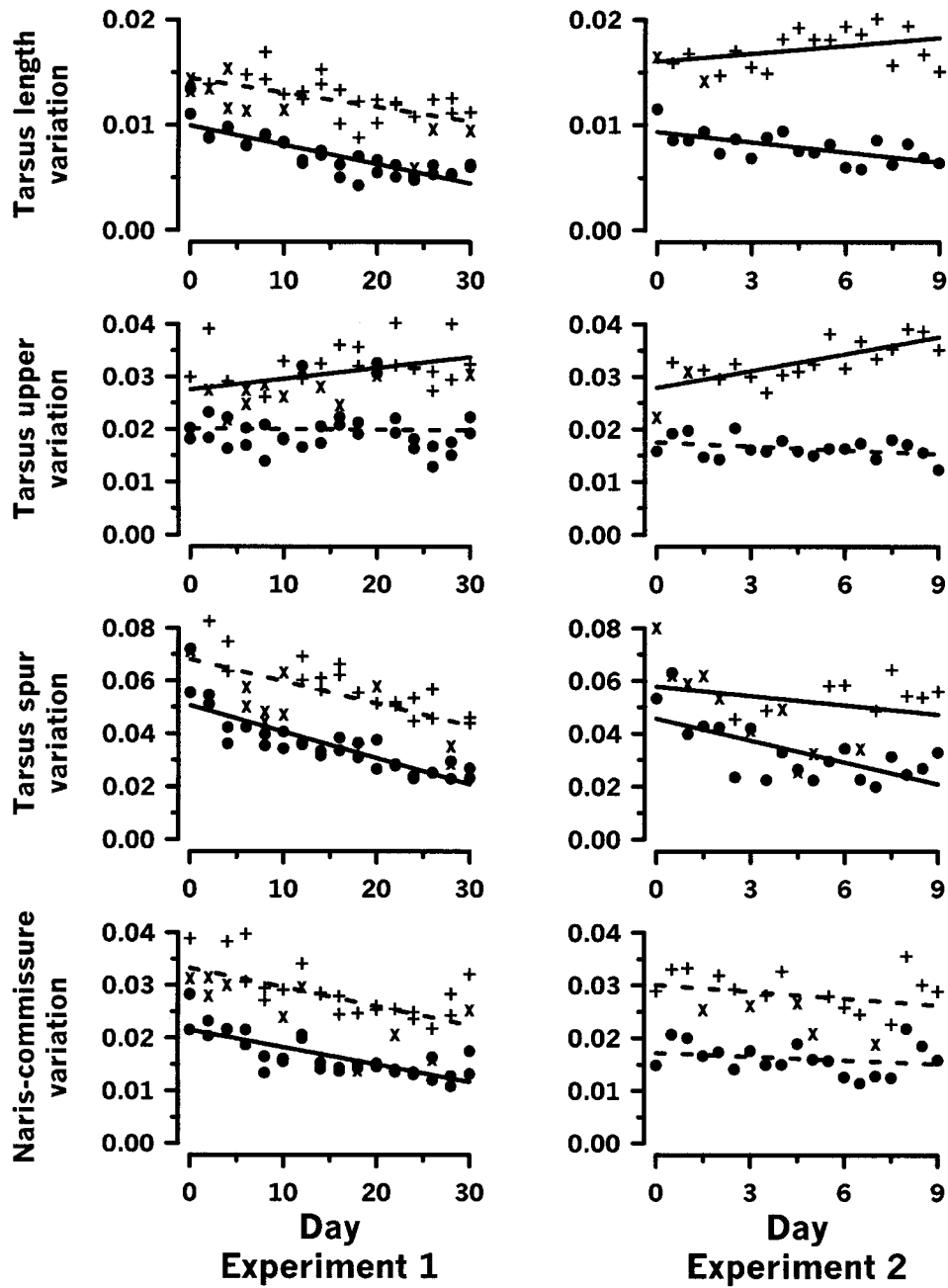


Figure 3: Plots of measurement error (ME2; Palmer and Strobeck 2003; *filled circles*) and total asymmetry (as the sum of ME2 and FA10b; Palmer and Strobeck 2003; plus sign if FA10b is significantly greater than 0, x if not) for each character measured in each treatment at each time of measurement over the course of experiments 1 and 2. Fluctuating asymmetry (FA) is the difference between the elevation of the total asymmetry and ME2 points for each day. Regression lines indicate trends and are solid if the Spearman rank correlation of ME2 or FA10b with day was significant or dashed if it was not. Units are natural logarithm of length in mm. Fluctuating asymmetry was significantly greater than 0 on many days for each character in both experiments. Measurement error tended to decline over time in both experiments, while FA in three of the four characters remained nearly constant over the longer periods of experiment 1 and increased slightly with time in three of the four characters over the shorter, more intensive periods of experiment 2. This suggests that in most characters, FA levels increase early in growth, then remain constant, while ME decreases throughout growth.

(210.0 ± 22.6 g [mean ± SD], $n = 16$) was significantly less ($t = 16.8$, $df = 38$, $n = 40$, $P < .001$) than that of the control group (377.2 ± 35.2 g, $n = 24$). However, we detected no significant differences in asymmetry score values between control and hunger-stressed groups using any of the three indices (CFA 1: $t = 1.397$, $df = 38$, $P = .170$; CFA 2: $t = 0.000$, $df = 38$, $P = 1.00$; CFA 3: $t = -0.032$, $df = 38$, $P = .975$), even before Bonferroni adjustment.

At the time of final measurement in the second experiment, after 38–39 d of growth, the mean masses of pullets reared under high- and low-density conditions were 317.3 ± 73.7 g ($n = 19$) and 391.6 ± 49.8 g ($n = 14$), respectively. The mean mass of high-density birds was significantly less than the mean mass of low-density birds ($t = 3.260$, $df = 31$, $P = .003$). Asymmetry was not significantly greater than measurement error in two traits: the width of the tarsometatarsus joint and the distance from the naris to the commissure of the mandibles. We therefore excluded these traits from comparisons of high- and low-density birds; CFAs used in those analyses included only the length of the tarsometatarsus and the width of the tarsometatarsus at the spur. As in the first experiment, comparisons of asymmetry score values between high- and low-density birds detected no significant difference between the treatments with any index (CFA 1: $t = 1.714$, $df = 31$, $P = .096$; CFA 2: $t = 1.501$, $df = 31$, $P = .144$; CFA 3: $t = 1.659$, $df = 31$, $P = .107$), even before Bonferroni adjustment of significance levels.

In all the analyses we report subsequently, as well as the analyses reported above, we first compared outcomes between animals in the stressed and nonstressed treatments. These groups never differed significantly or substantially for any parameter or in any analysis. All of the results we subsequently report are therefore the results for analyses that combined the data for stressed and nonstressed animals.

Spectral Decompositions and Long-Term Patterns of Autocorrelation

The spectral decompositions could not differentiate the time series of asymmetry of most individuals from white noise in both experiments (table 2). This indicates that the time series did not show regular oscillations.

Short-Term Relationships between Asymmetry and Changes in Asymmetry

Investigations of the ACFs between levels of asymmetry and changes between measurements within traits in the first experiment revealed significant negative relationships between A_t and future changes in asymmetry; A_t was sig-

nificantly negatively correlated with $\Delta A_{0,48}$ for all four traits (table 3). Much weaker negative relationships were detected between A_t and $\Delta A_{48,96}$ for three traits and between A_t and $\Delta A_{96,144}$ for two traits. Only the relationships between A_t and $\Delta A_{0,48}$ accounted for more than very small proportions of the variation in the data (table 3).

In the second experiment, investigations of the relationships between A_t and lagged changes between measurements within traits revealed significant negative relationships between A_t and $\Delta A_{0,12}$ for all four traits (table 3). Two traits exhibited significant negative relationships between A_t and $\Delta A_{12,24}$ and A_t and $\Delta A_{24,36}$ were significantly correlated for only one trait.

We compared the correlations of A_t with $\Delta A_{0,48}$ between experiments 1 and 2 using a t -test for each character; none showed any significant difference even before Bonferroni adjustment (all $P \gg .05$), and the means of the correlations of all were very similar between the two experiments (table 3). This suggests that similar processes caused the relationship between A_t and $\Delta A_{0,48}$ in both experiments.

When we statistically controlled for the effect of $A_{t+\tau}$, partial ACFs for all traits in both experiments revealed very weak relationships between A_t and the change in asymmetry from $t + \tau$ to $t + 2\tau$ ($\Delta A_{t+\tau, t+2\tau}$; fig. 4) that did not differ significantly from 0.

Compensational Changes in Growth Rate

We examined the relationship between A_t and the relative acceleration or deceleration of growth on each side of the body between measurements by examining correlations between A_t and residuals from expected growth increments predicted by regressions of change in size on size of structure fitted for each character on each side of each individual. The mean correlations between A_t and the residual of change of each character on each side of the body and the results of the t -tests to determine whether these means differ significantly from 0 are summarized in table 4. These analyses are conservative with respect to the slight amounts of directional asymmetry exhibited by most structures (fig. 2), which would have tended to decrease the significance of correlations. They indicate that all structures in both experiments showed compensatory growth.

Comparing Parameters of Simulated and Real Time Series

We used box plots to compare the distributions of three types of correlations between real and simulated data sets. The correlations we examined were simple ACFs between A_t and $\Delta A_{t, t+\tau}$ and between A_t and $\Delta A_{t+\tau, t+2\tau}$. We also

Table 2: Summary of the results of spectral decompositions carried out on each time series of each trait for each individual in experiments 1 and 2

Trait	Number of Tests		Number of tests significantly different from white noise (Bonferroni $P < .05$)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Tarsometatarsus length	40	20	0	0
Tarsometatarsus spur	40	20	0	3
Tarsometatarsus joint	40	20	4	1
Naris-jaw commissure	40	20	2	0

examined partial ACFs between A_t and $\Delta A_{t+\tau, t+2\tau}$ by removing the effect of $A_{t+\tau}$ (fig. 4). In most cases, the distributions of correlations calculated for the real and simulated data sets were very similar, with medians and upper and lower quartile boundaries in similar positions. The ranges of distributions of correlations for simulated data were greater than those for real data. The exception to this is the simulations that used four iterations of the parameters estimated for the second experiment (12-h intervals) to create each new data point, thus emulating measurements taken over a longer timescale on a process operating at a shorter timescale. The ranges of the correlations estimated for these simulations were similar to the ranges of correlations for the real data (fig. 4).

Discussion

Measurements of asymmetry have often been used as indices of developmental instability (reviewed by Møller and Swaddle [1997]). Despite this, little is known regarding the developmental origins of asymmetry. Although many hypotheses regarding those origins can be identified in the literature (Grüneberg 1935; Waddington 1957; Van Valen 1962; Soulé 1982; Chippendale and Palmer 1993; Emlen et al. 1993; Hallgrímsson 1993, 1998, 1999; Swaddle and Witter 1997), no study has compared the predictions of all of them to a single set of data. Understanding the developmental origins of asymmetry is particularly important when it is used as an indicator of environmental stress. Using present knowledge, it is impossible to determine what temporal pattern of stress has led to observed levels of asymmetry. Elevated levels of asymmetry could result from a discrete episode of stress early in ontogeny that has derailed the normal developmental pathway and has since subsided (directional external cues, magnification of asymmetry, persistent asymmetry, and accumulation of accidents hypotheses), or it could be the product of ongoing present-day stress (accumulation of accidents, compensatory growth, and residual asymmetry hypotheses). Reconstructing the likely causes of elevated asym-

metry in stressed populations would be greatly simplified if the developmental origins of asymmetry were better understood. We identified seven hypotheses that attempt to explain the ontogeny of asymmetry in individuals. Our results allow us to discriminate between these hypotheses.

Comparing the Hypotheses with Our Data

The directional external cues hypothesis suggests that a sided environmental influence can induce asymmetry (Grüneberg 1935; Hallgrímsson 1993, 1998, 1999; fig. 1A). This hypothesis may explain the strong directional asymmetry we observed in the first few days posthatching, which could have been caused by the contortion of chicks within eggs. This asymmetry rapidly decreased, and the patterns of development we observed in individual chicks are clearly inconsistent with those predicted by this hypothesis as it is usually stated (table 1). It is, however, possible that short-term directional external cues that fluctuate in intensity and direction over time may serve as one source of the developmental noise that causes departures from symmetry that are subsequently corrected.

The coin-toss and accumulation of accidents hypotheses predict that the degree and direction of changes in asymmetry will be random (Soulé 1982; Hallgrímsson 1993, 1998, 1999; fig. 1; table 1). In our study, changes in asymmetry between measurements were not random but were a function of the magnitude of previous asymmetry, with larger asymmetry values preceding relatively larger changes in the opposite direction than smaller asymmetry values. We therefore reject the coin-toss hypothesis and the accumulation of accidents hypothesis as adequate explanations of the ontogeny of asymmetry in the domestic fowl.

We can also reject the magnification of asymmetry and persistent asymmetry hypotheses. These hypotheses suggest that asymmetries can arise in the early stages of growth and development and persist in sign and magnitude throughout ontogeny (Chippendale and Palmer 1993; Emlen et al. 1993; Hallgrímsson 1993, 1998, 1999; fig. 1; table 1). Visual examination of our individual asymmetry time

Table 3: Summary of analyses examining correlations between asymmetry of measured structures at time t (A_t) and change in asymmetry (ΔA) over three measurement intervals for experiments 1 and 2

A_t for structure and change	Mean correlation	(Mean correlation) ²	SD	t	$P(t)$
Experiment 1 ($N = 40$):					
Tarsus length:					
Δ_{0-48}	-.656	.431	.125	-33.24	<.0001
Δ_{48-96}	-.082	.007	.210	-2.47	.0181
Δ_{96-144}	-.036	.001	.218	-1.03	.3071
Tarsus width at spur:					
Δ_{0-48}	-.659	.435	.115	-36.22	<.0001
Δ_{48-96}	.067	.004	.253	1.67	.1020
Δ_{96-144}	-.088	.008	.256	-2.17	.0364
Tarsus upper width:					
Δ_{0-48}	-.568	.323	.102	-35.13	<.0001
Δ_{48-96}	-.168	.028	.223	-4.77	<.0001
Δ_{96-144}	-.129	.017	.204	-4.00	.0003
Naris-jaw commissure:					
Δ_{0-48}	-.640	.410	.124	-32.56	<.0001
Δ_{48-96}	-.053	.003	.213	-1.58	.1218
Δ_{96-144}	.040	.002	.260	.98	.3338
Experiment 2 ($N = 20$):					
Tarsus length:					
Δ_{0-12}	-.524	.274	.090	-25.88	<.0001
Δ_{12-24}	-.155	.024	.223	-3.12	.0057
Δ_{24-36}	-.076	.006	.150	-2.25	.0362
Δ_{0-48}	-.756	.572	.142	-23.68	<.0001
Tarsus width at spur:					
Δ_{0-12}	-.600	.360	.138	-19.41	<.0001
Δ_{12-24}	-.087	.008	.211	-1.85	.0800
Δ_{24-36}	-.014	.000	.231	-.27	.7875
Δ_{0-48}	-.719	.517	.263	-12.25	<.0001
Tarsus upper width:					
Δ_{0-12}	-.607	.368	.111	-24.36	<.0001
Δ_{12-24}	-.008	.000	.222	-.16	.8751
Δ_{24-36}	-.113	.013	.254	-1.99	.0607
Δ_{0-48}	-.596	.355	.475	-5.62	<.0001
Naris-jaw commissure:					
Δ_{0-12}	-.644	.415	.099	-29.17	<.0001
Δ_{12-24}	-.123	.015	.217	-2.54	.0200
Δ_{24-36}	-.058	.003	.194	-1.33	.1987
Δ_{0-48}	-.684	.468	.278	-11.02	<.0001

series (fig. 2) showed that the signs and magnitudes of asymmetries changed throughout ontogeny. The magnification of asymmetry and persistent asymmetry hypotheses can therefore be rejected.

The hypothesis of compensatory growth states that symmetry is maintained by feedback between corresponding right and left structures that enables catch-up growth (Waddington 1957; Emlen et al. 1993; fig. 1G). This could occur by restraining the rate of growth on the larger side or hastening growth on the lagging side, which could lead to oscillations in asymmetry between sides (Emlen et al.

1993). Our data demonstrate that individuals corrected asymmetries by both increasing the growth rate of the lagging side and reducing the rate of growth on the larger side. These corrections did not, however, lead to regular oscillations in asymmetry. The hypothesis that symmetry is maintained by oscillations between right and left structures can therefore be rejected for domestic fowl, but the compensatory growth hypothesis is otherwise an adequate description of the processes that give rise to asymmetry patterns in this species.

The results of this study closely fit the expectations of

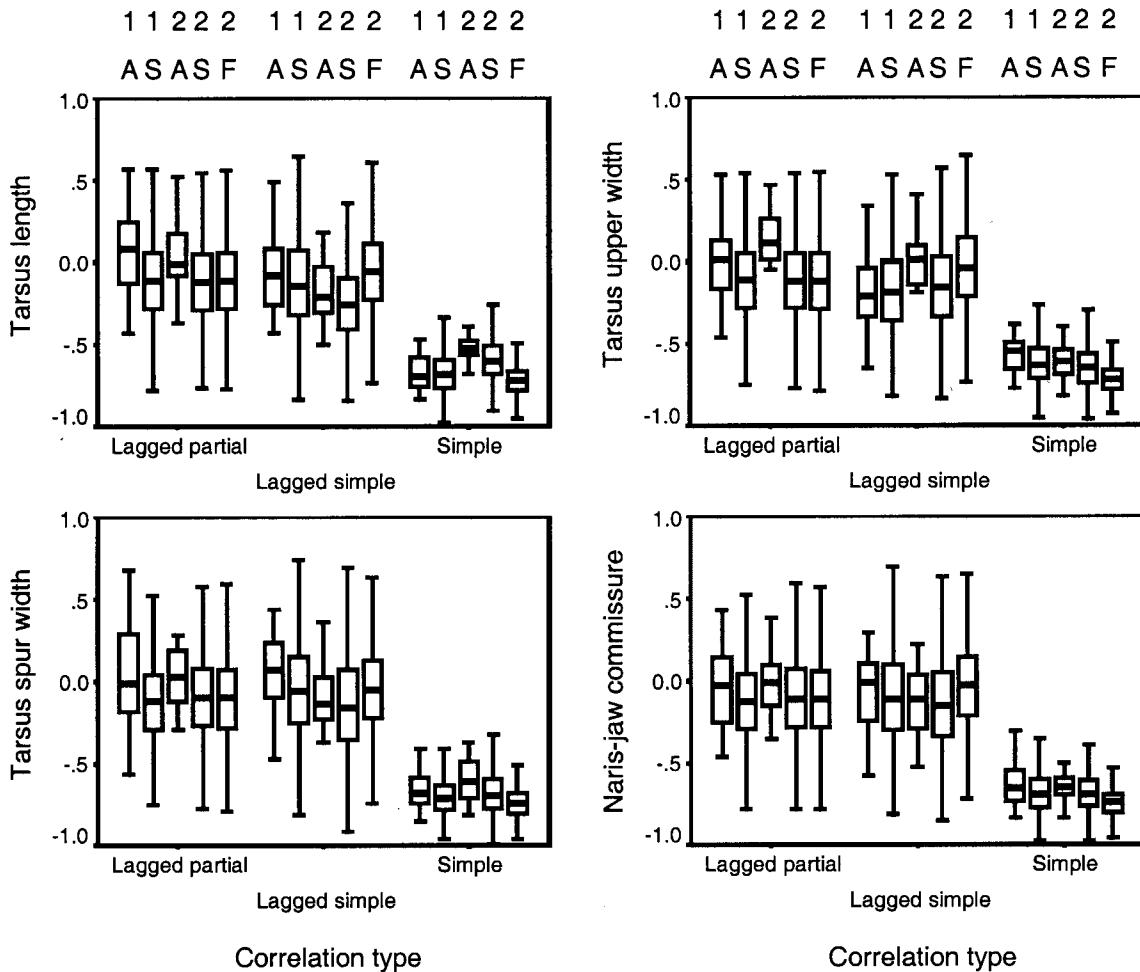


Figure 4: Box plots (Tukey 1977) showing medians, ranges, and quartiles for correlations between A_t and $\Delta A_{0-\tau}$ (simple), A_t and $\Delta A_{\tau-2\tau}$ (lagged simple), and A_t and $\Delta A_{\tau-2\tau}$ with the effect of $A_{+\tau}$ removed (lagged partial). Data are from birds measured in experiments 1 and 2 (1A, 2A), birds simulated using parameters estimated from experiments 1 and 2 (1S, 2S), and birds simulated using parameters from experiment 2, but with measurements taken every 48 h, as in experiment 1 (2F).

the residual asymmetry hypothesis. Van Valen (1962) predicted that fluctuating asymmetry is simply what is left over after the animal has “tried” to correct as much as possible for noise-induced departures from the pathway of development (fig. 1H). Examinations of patterns in individual asymmetry time series indicated that relative asymmetry increased early in ontogeny and then tended to remain constant for the remainder of growth (fig. 2). Levels of FA behaved similarly in the populations measured in each treatment in each experiment at each time (fig. 3). Our analyses of short-term temporal structure within individual asymmetry time series showed that individuals corrected for perturbations to development soon after they occurred by changing the relative rates of growth on both sides so as to reduce asymmetry.

We were able to produce AR(1) simulation models that very closely mirrored the behavior of asymmetry time series in chickens by incorporating only two processes: normally distributed perturbations random in both degree and direction, simulating “developmental noise,” and short-term compensatory corrections dependent only on the degree of asymmetry in the previous time period. It appears that although most of the behavior of real time series may be explained by this simple process, there are also weaker but more complex mechanisms at work. The partial correlations between asymmetry at time t and the change in asymmetry from time $t + \tau$ to $t + 2\tau$ were weakly positive but not significant for the measured data and weakly negative for the simulated data (fig. 4), suggesting that there are longer-term mechanisms in opera-

Table 4: Summary of analyses examining correlations between A_i and the residual of change in size of structure from a regression of change in size from t to $t + \tau$ on size at t for each structure on each side of each individual

Character and side	Mean r	SD	t	$P(t)$
Experiment 1 ($N = 40$):				
Tarsus length:				
Left	.352	.231	9.651	<.0001
Right	-.234	.221	-6.709	<.0001
Tarsus width at spur:				
Left	.439	.173	16.079	<.0001
Right	-.365	.199	-11.574	<.0001
Tarsus upper width:				
Left	.340	.218	9.851	<.0001
Right	-.250	.231	-6.851	<.0001
Naris-jaw commissure:				
Left	.466	.215	13.697	<.0001
Right	-.327	.248	-8.329	<.0001
Experiment 2 ($N = 20$):				
Tarsus length:				
Left	.244	.152	7.183	<.0001
Right	-.361	.150	-10.742	<.0001
Tarsus width at spur:				
Left	.363	.201	8.062	<.0001
Right	-.152	.200	-3.392	<.0001
Tarsus upper width:				
Left	.327	.203	7.198	<.0001
Right	-.326	.155	-9.372	<.0001
Naris-jaw commissure:				
Left	.257	.150	7.634	<.0001
Right	-.449	.167	-11.997	<.0001

Note: Because A_i is signed $R_i - L_i$, positive correlations for structures on the left side of the body and negative correlations for structures on the right side of the body both indicate that compensatory growth occurs.

tion. The average magnitudes of these correlations were very small, however, with a maximum (mean r)² of 0.016. This suggests that although higher-order mechanisms may operate, they do not have strong effects. Most corrections appear to occur on very short timescales.

Our finding that deviations from the normal pathway of development are subsequently corrected during growth is in contrast to Hallgrímsson (1998, 1999), who found evidence that FA increases ontogenetically in skeletal series of humans (*Homo sapiens*) and rhesus macaques (*Macaca mulatta*). One way to reconcile this apparent contradiction is to note that a number of mechanisms probably act to produce FA and that ontogenetic patterns in asymmetry at different scales could be products of fundamentally different processes. For example, although our data did not support the directional external cues hypothesis, it is very unlikely that sided mechanical stimuli do not play some part in inducing asymmetries (Hallgrímsson 1999). It seems possible that short-term compensatory mechanisms

could be important at small scales, but over lifetimes in long-lived organisms, there could be a trend for accidents to accumulate through processes such as bone turnover. Given the low rates of FA accumulation documented by Hallgrímsson (1999), this seems a likely explanation.

Asymmetry and Stress Relations

It was surprising that we found no effects of stress on levels of asymmetry in either experiment, despite clear differences in growth rates between groups. Since the domestication of wild jungle fowl 9,000 years ago, humans have selected both actively and passively for strains with desired characteristics (Simm et al. 1996). It seems possible that in doing so, types that were capable of high precision in development (i.e., producing a desired state at a desired time) or resistant to the stress often encountered in captivity may have been favored, resulting in the high levels of stability in the face of stress exhibited by the chickens in this study.

We are not aware of any other work on FA-stress relations that has attempted to reproduce the results of a previous study. Møller et al. (1995) detected a significant positive relationship between increases in rearing density from 20 to 24 and 28 chickens per square meter and increases in fluctuating asymmetry in two fast-growing breeds of chickens (ScanBrid and Ross 208). Our study did not detect differences between asymmetry scores of chickens reared at densities of 12 and 30 birds per square meter.

One explanation for these contradictory results could be that breeds differ in their ability to resist stress-induced deviations from symmetry. Møller et al. (1995) noted that La-Belle Rouge chickens, which are slow-growing chickens, might be better able to resist stress than fast-growing Ross 208 or ScanBrid animals. There might be differences in developmental instability among fast-growing breeds as well. The conflicting results could also be attributed to differences in the absolute sizes of chicken pens. The pens used by Møller et al. (1995) were 37.5 m² and contained several hundred chickens, while this study used pens that were 0.17 m² with only two or five chickens in each pen. It is possible that density stress is insufficient to elevate levels of FA unless it is coupled with large numbers of individuals, which could cause extreme local crowding, for example, around food or water sources. If this is the case, the level of social stress in a pen that contained many individuals could be much greater than one would predict given the mean density. However, reasonably large and significant differences in growth rates were detected between high and low densities in this study, indicating that we had produced substantial density effects that affected

traditional fitness measures (body mass) but not asymmetry, a result also found by McCoy and Harris (2003) in a study of the salamander *Ambystoma maculatum*.

Application to Conservation Biology

Several authors have suggested that monitoring levels of fluctuating asymmetry in wild or captive populations could provide a warning of increases in stress before declines in population numbers (e.g., Leary and Allendorf 1989; Clarke 1995; Alford et al. 1999). The interpretation of differences in asymmetry levels among populations or times depends on how the asymmetry of individuals changes through growth and development. Our results show that asymmetry in domestic fowl is not the product of events over their entire growth history but is influenced mainly by developmental noise in the recent past. This suggests that elevated levels of asymmetry within a population are likely to result from a present or very recent stress and are not the cumulative effects of previous stresses or the effects of stress during a critical stage in early development. Fluctuating asymmetry could therefore be useful as a monitoring tool because present levels of asymmetry are not confounded by events in a population's growth history, which is important when trying to assess present conditions. However, in at least some populations of some species, our results suggest that even moderately severe environmental stresses may not affect FA levels, so that for it to be of use as a monitoring tool, something must be known of the responses of FA to stress in the taxon or taxa of interest.

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