Inbreeding reduces power-law scaling in the distribution of fluctuating asymmetry: an explanation of the basis of developmental instability

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The study of fluctuating asymmetry has been controversial because of conflicting results found in much of the primary literature. It has been suggested that the source of this conflict is the fact that the basis of fluctuating asymmetry is poorly understood and that, as a consequence, methodology of fluctuating asymmetry studies may be flawed. A new model for the phenomenological basis of fluctuating asymmetry, that variation in fluctuating asymmetry is in large part due to the random exponential growth of cell populations (geometric Brownian motion) that are terminated randomly around a genetically programmed development time, is presented here. If termination of development has a genetic component, then scaling effects and kurtosis in the distribution of fluctuating asymmetry should increase with genetic redundancy of the population. This model prediction was tested by comparing the distribution of multivariate size and shape fluctuating asymmetry in large samples collected from

both wild populations and four moderately inbred lines of *Drosophila simulans*. It was found that while wild populations were best described by a lognormal distribution with power-law scaled tails, the inbred lines derived from the wild stock were dramatically normalized (half-normal) in three of four cases. As predicted, the scaling exponent of the upper tail of the distribution of fluctuating asymmetry increased with inbreeding while the kurtosis and mean fluctuating asymmetry decreased with inbreeding. The model suggests an additional explanation of leptokurtosis in fluctuating asymmetry. Kurtosis and scaling of the statistical distribution of fluctuating asymmetry in a population is related directly to genetic differences between individuals and these differences affect their ability to buffer the process of development against random perturbations.

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Introduction

The study of fluctuating asymmetry, a measure of developmental instability, has a controversial history. Fluctuating asymmetry is hypothesized by some to reflect a population's response to environmental or genetic stress (Graham, 1992; Parsons, 1992; Clarke, 1993). It is also generally accepted that fluctuating asymmetry may act as an indicator or signal of individual genetic buffering capacity to environmental stress (Møller, 1990; Møller and Pomiankowski, 1993; Møller and Swaddle, 1997). Recent literature reviews reveal that these conclusions are perhaps premature and that individual studies often demonstrate conflicting results (Bjorksten *et al*, 2000; Lens *et al*, 2002). Babbitt *et al* (2006) demonstrate that conflicting results of many individual studies may be caused by under-sampling due to the false assumption that fluctuating asymmetry always exhibits a normal distribution. Also, fluctuating asymmetry may be responding to experimental treatment in a complex and as yet unpredictable fashion. Until the basis of fluctuating asymmetry is better understood, general interpretation of studies remains difficult.

What is the basis of fluctuating asymmetry?

Theoretical attempts have been made to explain how fluctuating asymmetry may be generated during development and some predictions of theory have been recently tested (Swaddle and Witter, 1997; Tompkin, 1999; Freeman *et al*, 2003; Kellner and Alford, 2003). Models for the phenomenological basis of fluctuating asymmetry fall into two general categories: reaction-diffusion models and diffusion-threshold models (reviewed in Klingenberg, 2003). The former class of models involves the chaotic and nonlinear dynamics in the communication among neighboring cells (Emlen *et al*, 1993) or chaotic and nonlinear dynamics created by paired Turing models representing morphogen and inhibitor activity in adjacent bilateral morphology (Graham *et al*, 1993). The latter class of models combines morphogen diffusion and a threshold response, the parameters of which are controlled by hypothetical genes and a small amount of random developmental noise (Klingenberg and Nijhout, 1999). The result of this latter class of model is that different genotypes respond differently to the same amount of noise, providing explanation for genetic variation in fluctuating asymmetry response to the same environments.

Traditionally, models for the proximate basis of fluctuating asymmetry assume that variation in fluctuating asymmetry arises either from independent stochastic events that accumulate over time or from nonlinear

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dynamics in the regulation of growth. None of these traditional models explicitly or mathematically address the affect of stochastic behavior in cell cycling during exponential growth. More recently Graham *et al* (2003a) make a compelling argument that fluctuating asymmetry often results from multiplicative errors during growth. This is consistent with one particular detail about how cells behave during growth. For several decades there has been evidence that during development, cells actually compete to fill prescribed space until limiting nutrients or growth signals are depleted (Day and Lawrence, 2000; Diaz and Moreno, 2005). So cell populations effectively double each generation until signaled or forced to stop. It has also been observed that in *Drosophila* wing disc development, that synchrony (or phase locking) in cell cycling does not occur across large tissue fields but rather extends only to an average cluster of 4–8 neighboring cells regardless of the size and stage of development of the imaginal disc (Milan *et al*, 1996). The assumption of previous models, particularly the reaction-diffusion type, that cell populations are collectively controlling their cell cycling rates across a whole developmental compartment is probably unrealistic. Regulatory control of fluctuating asymmetry almost certainly does occur, but probably at a higher level involving multiple developmental compartments where competing cells are prevented from crossing boundaries. However, given that individual cells are behaving more or less autonomously during growth within a single developmental compartment, it is suggested that variation in fluctuating asymmetry can be easily generated at this level by a process related to stochastic exponential expansion and its termination in addition to regulatory interactions that probably act at higher levels in the organism. This paper explores and tests simple model predictions regarding the generation of fluctuating asymmetry through multiplicative error without regulatory feedback.

Exponential growth and non-normal distribution of fluctuating asymmetry

In previous work, Babbitt *et al* (2006) demonstrate that the distribution of unsigned fluctuating asymmetry best fits a lognormal distribution with power-law tails (double Pareto-lognormal distribution). This distribution is similar to lognormal but has fatter tails that dissipate more slowly. This distribution can be generated by random exponential growth (ie geometric Brownian motion) that is stopped or observed randomly according to a negative exponential probability (Reed, 2001; Reed and Jorgensen, 2004). It is suggested that this potential source of power-law scaling in the tails of the fluctuating asymmetry distribution is also a potential cause of some of the leptokurtosis that is often observed empirically in the distribution of fluctuating asymmetry (noted in Gangestad and Thornhill, 1999; Graham *et al*, 2003a). Kurtosis is the value of the standardized fourth central moment. Like the other moments, (location, scale, and skewness), kurtosis is best viewed as a vague concept that can be formalized in multiple ways (Mosteller and Tukey, 1977). Leptokurtosis is best visualized as the location and scale-free movement of probability mass from the shoulders of a symmetric distribution towards both its center and tail (Balandia and MacGillivray, 1988).

Both the Pareto and power-function distributions have shapes characterized by the power-law and a large tail and therefore exhibit a total lack of characteristic scale. Both because kurtosis is strongly affected by tail behavior, and because leptokurtosis involves a diminishing of characteristic scale in the shape of a distribution, the concepts of scaling and kurtosis in real data can be, but are not necessarily always, interrelated.

Leptokurtosis in the distribution of fluctuating asymmetry has often been attributed to a mixture of normal fluctuating asymmetry distributions caused by a mixing of individuals, all with different genetically based developmental buffering capacity, or in other words, different propensity for expressing fluctuating asymmetry (Gangestad and Thornhill, 1999; Palmer and Strobeck, 2003; Van Dongen *et al*, 2005). Although not noted by these authors, the Laplace distribution is represented as a continuous mixture of normal distributions (Kotz *et al*, 2001; Kozubowski and Podgorski, 2001) and can be distinguished from other potential candidate distributions by using appropriate model selection techniques, such as the Akaike Information Criterion technique (Burnham and Anderson, 1998). Graham *et al* (2003a) rejects the typical explanation of leptokurtosis through the mixing of normal distributions by noting that differences in random lognormal variables can generate leptokurtosis. Babbitt *et al* (2006) also rejects the explanation that leptokurtosis in the distribution of fluctuating asymmetry is caused by a mixture of normal distributions because the double Pareto-lognormal distribution, and not the Laplace distribution, always appears the better fit to large samples of fluctuating asymmetry. Therefore, leptokurtosis often observed in the distribution of fluctuating asymmetry is probably not always caused by a mixing process, but instead may be an artefact of non-normal distribution in fluctuating asymmetry. Furthermore, because double Pareto-lognormal distribution can be generated by geometric Brownian motion, its presence, could support evidence of multiplicative error during exponential expansion of populations of cells.

Testing a model for the basis of fluctuating asymmetry

It is proposed that the proximate basis for variation of fluctuating asymmetry in a population of organisms is due to the random termination of stochastic geometric growth. The combination of opposing stochastic exponential functions results in the slow power-law decay that describes the shape of the distribution's tail. This paper presents a generative model for fluctuating asymmetry and through simulation, tests the prediction that variation in the ability to precisely terminate growth will lead to increased kurtosis and decreased scaling exponent in the upper tail of the distribution. Then the validity of this model is assessed by direct comparison to the distribution of fluctuating asymmetry within large samples ($n = 1000$) of wild and inbred populations of *Drosophila simulans*. Under the assumption that a population with less genetic variation will also exhibit less temporal variation in the termination of growth across the population, it can be predicted that inbreeding, which reduces variation through the reduction of heterozygosity and random fixation of alleles by drift, should act to reduce power-law scaling effects in the

distribution of fluctuating asymmetry in a population. Inbreeding should also reduce the tail weight (kurtosis) and mean fluctuating asymmetry assuming inbred individuals have lower variance in the times at which they terminate growth. It is also assumed that inbreeding within specific lines does not act to amplify fluctuating asymmetry due to inbreeding depression. It has been demonstrated that *Drosophila melanogaster* do not increase mean fluctuating asymmetry in response to inbreeding (Fowler and Whitlock, 1994) and it is suggested that large panmictic populations typical of *D. melanogaster* may not harbor as many hidden deleterious recessive mutations as other species (Houle, 1989), making them resistant to much of the typical genetic stress of inbreeding. Therefore, the absence of specific gene effects during inbreeding suggests that *Drosophila* may be a good model for investigating the validity of this model as an explanation of the natural variation occurring in population-level fluctuating asymmetry.

Methods

Model development

Simulation of geometric Brownian motion: Ordinary Brownian motion is most easily simulated by summing independent Gaussian distributed random numbers or white noise (X_i) (See Figure 1).

$$W(X) = \sum_{i=1}^n X_i \quad (1)$$

which simulated in discrete steps is

$$N_t = N_{t-1} + W_{t-1} \quad (2)$$

where N is the cell population size, t the time step and W is a random Gaussian variable.

Exponential or geometric Brownian motion, a random walk on a natural log scale, can be similarly simulated. Geometric Brownian motion is described by the stochastic differential equation

$$dY(t) = \mu Y(t)dt + vY(t)dW(t) \quad (3)$$

or also as

$$\frac{dY(t)}{Y(t)} = \mu dt + v dW(t) \quad (4)$$

where $W(t)$ is a Brownian motion (or Weiner process) and μ and v are constants that represent drift and volatility, respectively. See Reed and Jorgensen (2004). Equation (3) has an analytic solution:

$$Y(t) = Y(0)e^{(\mu - v^2/2)t + vW(t)} \quad (5)$$

A simulation of geometric Brownian motion in discrete form follows as

$$N_t = N_{t-1} + N_{t-1}W_{t-1} \quad (6)$$

where N is the cell population size, t is the time step and W is a random Gaussian variable (See Figure 2). Equation (6) is identical to the equation for multiplicative error in Graham *et al* (2003a). The Equation (6) is slightly modified by letting W range uniformly from 0.0 to 1.0 with $\bar{W} = 0.5$ and adding a drift constant C that allows for stochastic geometric or exponential growth (upward drift at $C > 0.5$) or decline (downward drift at $C < 0.5$).

$$N_t = CN_{t-1} + N_{t-1}W_{t-1} \quad (7)$$

At $C = 0.5$, Equations (6) and (7) behave identically. Geometric Brownian motion with upward drift is shown in Figure 3.

Figure 1 Ordinary Brownian motion (lower panel) in N simulated by summing independent uniform random variables (W)(upper panel). This was generated using Equation (2).

Figure 2 Geometric Brownian motion in N and $\log N$ simulated by multiplying independent uniform random variables. This was generated using Equation (5) with $C = 0.54$.

Simulation of fluctuating asymmetry: Using MathCad 2001 Professional Edition (Mathsoft Engineering and Education), two independent geometric random walks were performed and stopped randomly at mean time $t = 200$ steps with some variable normal probability. The random walks result in cell population size equal to N_t (or N_t^L and N_t^R on left and right sides, respectively). Fluctuating asymmetry (FA) was defined as the difference in size resulting from this random proportional growth on both sides of the bodies axis of symmetry plus a small degree of random uniformly distributed noise or

$$FA = N_t^L - N_t^R + rv \quad (8)$$

where (rv) was uniformly distributed with a range of $\pm 0.1(N_t^L - N_t^R)$. Using a MathCad-based simulation in VisSim LE, the generation of individual fluctuating asymmetry values was repeated until a sample size of 5000 was reached. The random noise (rv) has no effect on the shape of the fluctuating asymmetry distribution but helps even out binning gaps in distribution tails. As rv is small in comparison to $N_t^L - N_t^R$, its effect is similar to that of measurement error (which would be normally distributed rather than uniform). Schematic representation of the simulation process for Reed and Jorgensen (2004) model and the simulation of fluctuating asymmetry are shown in Figures 4 and 5, respectively. The simulation of the distribution of fluctuating asymmetry was compared at normal standard deviation of termination of growth (t) ranging from $\sigma = 0.5, 0.8, 1.2, 3$ and 7 with a drift constant of $C = 0.7$ (Figure 6).

Figure 3 Geometric Brownian motion in N and $\log N$ with upward drift. This was generated using Equation (5) with $C = 0.60$.

Inbreeding experiment

In May 2004 in Gainesville, Florida, 320 free-living *D. simulans* were collected in banana-baited traps and put into a two-gallon glass jar and cultured on instant rice meal and brewer's yeast. After two generational cycles, 1000 individuals were collected in alcohol. This was repeated again in June 2005 with 200 wild-trapped flies.

Lines of inbred flies were created from the May 2004 wild population through eight generations of full-sib crosses removing an estimated 75% of the pre-existing heterozygosity (after Crow and Kimura, 1970). Initially, 10 individual pairs were isolated from the stock culture and mated in $\frac{1}{2}$ pint mason jars with media and capped with coffee filters. In each generation, and in each line, and to ensure that inbred lines were not accidentally lost though an inviable pairing, four pairs of F1 sibs from each cross were then mated in $\frac{1}{2}$ pint jars. Offspring from one of these four crosses were randomly selected to set up the next generation. Of the original 10 lines, only four remained viable after eight generations of full-sib crossing. These remaining lines were allowed to increase to 1000+ individuals in 1-quart mason jars and then were collected for analysis in 85% ethanol. This generally took about four generations (8 weeks) of open breeding. One completely isogenic line of *D. melanogaster* was obtained from Dr Marta Wayne, University of Florida and also propagated and collected.

All flies were cultured on rice meal and yeast at 29°C with 12:12 LD cycle in environmentally controlled chambers at the Department of Entomology and Nematology at the University of Florida. Wings were collected and removed from 1000 flies from each of the two samples of wild popu-

Figure 4 A model representation of Reed and Jorgensen's (2004) physical size distribution model. Variable negative exponentially distributed stopping times of random proportional growth (GBM with $C = 0.5$) create double Pareto-lognormal distribution of size.

lation and four samples of inbred lines and dry mounted on microscope slides. Specimens were dried in 85% ethanol, and then pairs of wings were dissected (in ethanol) and air-dried to the glass slides. Permunt was used to attach coverslips. This technique prevented wings from floating up during mounting, which might slightly distort the landmark configuration. Dry mounts were digitally photographed. All landmarks were identified as wing vein intersections on the digital images (eight landmarks on each wing). See Appendix A for landmark locations.

Morphometric analyses

Wing vein intersections were digitized on all specimens using TPSDIG version 1.31 (Rohlf, 1999). Specimens damaged at or near any landmarks were discarded. Fluctuating asymmetry was measured in two ways on all specimens using landmark-based multivariate geometric morphometrics. A multivariate size-based fluctuating asymmetry (FA 1 in Palmer and Strobeck, 2003) was calculated as absolute value of $(R-L)$ or just $R-L$ in signed fluctuating asymmetry distributions, where R and L are the centroid sizes of each wing (ie, the sum of the distances of each landmark to their combined center of mass or centroid location). In addition, a multivariate shape-based measure of fluctuating asymmetry known as the Procrustes distance was calculated as the square root of the sum of all squared Euclidean distances between each left and right landmark after two-dimensional

Procrustes fitting of the data (Bookstein, 1991; Smith *et al.*, 1997; Klingenberg and McIntyre, 1998; FA 18 in Palmer and Strobeck, 2003). Centroid size calculation, Euclidean distance calculation and Procrustes fitting were performed using Øyvind Hammer's Paleontological Statistics program PAST version 0.98 (Hammer, 2002). A subsample of 50 individuals from the fourth inbred line (pp4B3) was digitized five times to estimate measurement error. In these cases, measures of fluctuating asymmetry were taken as the average fluctuating asymmetry value of the five replicate measurements for each specimen. Percent measurement error was also computed as $(ME/\text{average } FA) \times 100$, where $ME = SD(FA1, FA2, FA3, FA4, FA5)$ (as per Palmer and Strobeck, 2003). For assessing whether measurement error (ME) interfered significantly with fluctuating asymmetry, a Procrustes ANOVA (in Microsoft Excel) was performed on the five replications of the 50-specimen subsample (Klingenberg and McIntyre, 1998). Any subsequent statistical analyses were performed using SPSS Base 8.0 statistical software (SPSS Inc.).

Model selection and inference

The fits of unsigned size fluctuating asymmetry to four distributional models (half-normal, lognormal, asymmetric Laplace, and double Pareto-lognormal (DPLN)) were compared in both simulated data and in the *Drosophila* lines, by calculating negative log likelihoods and Akaike Information Criteria (AIC) (Burnham and

Figure 5 A model representation of developmental instability. Normally variable stopping times of random proportional growth (GBM with $C = 0.7$) create double Pareto-lognormal distribution of size.

Anderson, 1998; Hilborn and Mangel, 1998). This method penalizes more complex models (those with more parameters) when selecting the best-fit distributional model for a given data set. Best-fitting parameters were obtained by maximizing the log-likelihood function for each model (Appendix B). The maximization was performed using the conjugate gradient method within unconstrained solve blocks in the program MathCad 2001 (Mathsoft Engineering and Education).

Results

Model simulation

The amount of variance in termination times related directly to levels of fluctuating asymmetry (ie low variance in termination time (t) gives low fluctuating asymmetry and *vice versa*). It was found that not only does amount of fluctuating asymmetry increase with increased variance in (t), but so do both kurtosis and the scaling affect in the distribution tails (scaling exponents decrease). In Figure 6, normal quantile plots of signed fluctuating asymmetry are shown for different standard deviations in the normal variation of the termination of growth of geometrically expanding cell populations. The degree of the S or sigmoidal shape in the plot indicates

level of leptokurtosis. The leptokurtosis in the quantile plot is reduced greatly with a decrease in the standard deviation of the normal variation in growth termination times.

Experimental results

Both mean unsigned size fluctuating asymmetry and shape fluctuating asymmetry decreased with inbreeding in all lines. It was also observed that the kurtosis of signed size fluctuating asymmetry and the skewness of both unsigned size and shape fluctuating asymmetry follow an identical trend. The trend was strongest in kurtosis, which decreased rapidly with inbreeding, indicating that, as predicted, changes in mean fluctuating asymmetry are influenced strongly by the shape and tail behavior of the distribution of fluctuating asymmetry (Table 1). Although wild populations on the whole, demonstrated increased mean, skew and leptokurtosis of fluctuating asymmetry compared to inbred lines, significant differences between populations were found in all distributional parameters, even between each of the inbred lines (ANOVA mean size fluctuating asymmetry for all lines; $F = 27.49$, $P < 0.001$; ANOVA mean size fluctuating asymmetry for inbred lines only; $F = 14.38$, $P < 0.001$; note shape fluctuating asymmetry also show

same result). Overall, inbred lines demonstrated lower kurtosis, just slightly above that expected from a normal distribution (Table 1). They also had lower mean fluctuating asymmetry. No significant differences were found with respect to these results according to sexes of flies. Figure 7 shows the distribution of fluctuating asymmetry and detrended normal quantile plots for the wild population, four inbred lines and one isogenic

line, respectively. As in the simulated data, the degree of the S shape in the plot indicated level of leptokurtosis. The S shape in quantile plot is reduced greatly with inbreeding and nearly disappears in the isogenic line.

Model selection and inference

The comparison of candidate distributional models of unsigned size-based fluctuating asymmetry demonstrated normalization associated with inbreeding in three of the four inbred lines (Table 1). In the remaining inbred line, the half-normal candidate model was a close second to the double Pareto-lognormal distribution. In the wild population samples, the distribution of fluctuating asymmetry was best described by the double Pareto-lognormal distribution, a lognormal distribution with scaling in both tails. In the best-fitting parameters of this distribution there was no observable trend in lognormal mean or variance across wild populations and inbred lines. The scaling exponent of the lower tail (β in Reed and Jorgensen, 2004) was close to one in all lines while the scaling exponent in the upper tail (α in Reed and Jorgensen, 2004) increased with inbreeding (Table 1). Both samples of the wild population demonstrated the lowest scaling exponent in the upper tail of the distribution. The low scaling exponents here indicate divergence in variance (2004 and 2005, where $\alpha < 3$) and in the mean (2004 only, where $\alpha < 2$). The inbred lines all show higher scaling exponents that are consistent with converging mean and variance. The isogenic *D. melanogaster* line demonstrated the highest scaling exponent and the most normalized distribution of unsigned fluctuating asymmetry.

Measurement error was 7.6% for shape-based fluctuating asymmetry and 13.0% for centroid size-based fluctuating asymmetry. In a Procrustes ANOVA (Klingenberg and McIntyre, 1998) the mean squares for the interaction term of the ANOVA ($MS_{\text{Interaction}}$) was highly significant at $P < 0.001$, indicating that variation in fluctuating asymmetry was significantly larger than variation due to measurement error.

Discussion

Revealing the genetic component of fluctuating asymmetry

Although it can be risky to infer process from pattern, the very similar results of both the modeling and the inbreeding experiment in *Drosophila* seem to suggest the presence of a scaling component in the distribution of

Figure 6 Simulated distributions of cell population size and fluctuating asymmetry for different amounts of variation in the termination of growth (variance in normally distributed growth stop time).

Table 1 Distribution parameters and model fit for multivariate fluctuating asymmetry in two wild populations and four inbred lines of *Drosophila simulans* and one isogenic line of *Drosophila melanogaster*

	Kurtosis (signed size FA)	Mean (unsigned size FA)	Mean (shape FA)	Scaling exponent in upper tail	Halfnormal	Lognormal	Skew Laplace	DPLN
Wild 2004	28.25 ± 0.12	5.73 ± 0.38	0.0223 ± 0.0003	1.49	90.567	323.616	2424.256	0
Wild 2005	45.85 ± 0.15	4.68 ± 0.19	0.0237 ± 0.0003	2.87	6.176	261.476	2645.371	0
Inbred line 1 (OR18D)	2.43 ± 0.11	3.27 ± 0.09	0.0183 ± 0.0002	5.09	0	103.507	2195.206	15.463
Inbred line 2 (OR18D3)	8.85 ± 0.15	4.24 ± 0.13	0.0212 ± 0.0002	3.10	11.542	222.869	2531.224	0
Inbred line 3 (PP4B2)	2.90 ± 0.15	3.31 ± 0.09	0.0184 ± 0.0002	4.63	0	130.254	2374.659	44.012
Inbred line 4 (PP4B3)	3.30 ± 0.15	3.72 ± 0.11	0.0213 ± 0.0003	5.78	0	227.865	2432.923	79.655
Isogenic (mel75)	0.55 ± 0.11	3.9 ± 0.10	0.0201 ± 0.0002	9.99	0	84.886	2383.148	101.381

Model fits are ΔAIC for unsigned centroid size fluctuating asymmetry (zero is best fit, highest number is worst fit).

Figure 7 Distribution of fluctuating asymmetry and detrended fit to normal for two samples of wild population collected in Gainesville, FL in summers of 2004 and 2005 and four inbred lines of *Drosophila simulans* derived from eight generations of full-sib crossing of the wild population of 2004. Also included is one isogenic line of *Drosophila melanogaster* (mel75).

fluctuating asymmetry that is caused by a random multiplicative growth process as suggested previously by Graham *et al* (2003a). This parameter appears to change with the genetic redundancy of the population. More specifically, the scaling exponent(s) of the upper tail (α) of the unsigned fluctuating asymmetry distribution, or outer tails of the signed fluctuating asymmetry distribution, are increased with inbreeding, causing more rapid power-law decay in the shape of the tail. This effect also reduces kurtosis and apparently normalizes the

distribution of fluctuating asymmetry in more inbred populations. Therefore, it appears that individual genetic differences in the capacity to control variance in the termination of random proportional growth (ie geometric Brownian motion) are responsible for determining the shape of the distribution of fluctuating asymmetry. In other words, leptokurtosis ($kurtosis > 3$) in signed fluctuating asymmetry distribution indicates genetic variability in the population while normality ($kurtosis = 3$) indicates genetic redundancy. This effect may also be explained in part by inadvertent selection against highly asymmetric individuals in the cultures, but it is unlikely that wild populations would be able to harbor more asymmetric individuals than lab cultures grown on an immediate food supply (where flight would not be necessary).

As leptokurtosis is very often observed in fluctuating asymmetry in many studies, genetic variability may potentially account for a large proportion of the variability observed in the fluctuating asymmetry of a given population. Observed differences or changes in fluctuating asymmetry are therefore not only a response of development to environmental stress, but clearly also can reflect inherent differences in the genetic redundancy of populations. The significantly different levels of fluctuating asymmetry among the inbred lines in this study, presumably caused by the random fixation of certain alleles, also suggests that there is a strong genetic component to the ability to buffer development against environmental noise.

It is also important to note that kurtosis may potentially be a much stronger indicator of fluctuating asymmetry than the distribution mean. The low scaling exponents found in the non-normal distributions of fluctuating asymmetry in the wild populations of *D. simulans* are capable of slowing and perhaps even stopping, the convergence on mean fluctuating asymmetry with increased sample size. As kurtosis is a fourth order moment, estimating it accurately also requires large sample sizes. However, if kurtosis can be demonstrated to respond as strongly to environmental stress as it does here to inbreeding, its potential strength as a signal of fluctuating asymmetry may allow new interpretation of past studies of fluctuating asymmetry without the collection of more data. This may help resolve some of the current debate regarding fluctuating asymmetry as a universal indicator of environmental health and as a potential sexual signal in 'good genes' models of sexual selection.

Limitations of the model

There are certain aspects of the model presented here that may be oversimplifications of the real developmental process. First of all, this model assumes developmental instability is generated by left-right growth of tissue fields with no regulatory feedback or control other than when growth is stopped. It is very likely that left-right regulation is able to occur at higher levels of organization (eg across multiple developmental compartments) even though there is no evidence of regulated cell cycling rates beyond the distance of 6–8 cells on average within any given developmental compartment (Milan *et al*, 1996). Therefore, this model explains how fluctuating asymmetry is generated, not how it may be

regulated. Second, this model only considers cell proliferation as influencing size. It is known that both cell size and programmed cell death, or apoptosis, are also important in regulating body size (Raff, 1992; Conlin and Raff, 1999). Both of these may play a more prominent role in vertebrate development, than they do in insect wings, where apparently growth is terminated during its exponential phase. Nevertheless, this simple model seems to replicate very well, certain dynamic aspects of the distribution of fluctuating asymmetry in the insect wing.

Potential application to cancer screening

Just as change in the shape of the distribution of fluctuating asymmetry is normalized across a population of genetically redundant individuals, genetic redundancy in a population of cells may also help maintain normal cell size and appearance. The loss of genetic redundancy in a tissue is a hallmark of cancer. The abnormal gene expression and consequent genetic instability that characterizes cancerous tissue often results in asymmetric morphology in cells, tissues and tissue borders. Baish and Jain (2000) review the many studies connecting fractal (scale free) geometry to the morphology of cancer. Cancer cells also are typically pleomorphic or more variable in size and shape than normal cells and this pleomorphy is associated with intercellular differences in the amount of genetic material (Ruddon, 1995). Frigesyi *et al* (2003) have demonstrated a power-law distribution of chromosomal aberrations in cancer. Currently, the type of distributional shape of the pleomorphic variability in cell size is not known, or at least not published. However, in 2001, Mendes *et al* (2001) demonstrated that cluster size distributions of HN-5 (cancer) cell aggregates in culture followed a power-law scaled distribution. Furthermore, these authors also demonstrated that in MDCK (normal) cells and Hep-2 (cancer) cells, cluster size distributions transitioned from short-tailed exponential distributions to long-tailed power-law distributions over time. The transition is irreversible and is likely an adaptive response to high density and long permanence in culture due to changes in either control of replication or also perhaps cell signaling. Taken collectively, these studies may suggest that scaling at higher levels of biological organization observed in cancer is due to increased relative differences in length of cell cycling rates of highly pleomorphic cell populations that have relatively larger intercellular differences in their amount of genetic material. The stochastic growth model has been proposed as the basis for higher variability in population level developmental instability or fluctuating asymmetry (Babbitt *et al*, 2006) may also provide a possible explanation for higher variability in both cell and cell cluster size of cancerous tissue. If genetic redundancy in growing tissue has the same distributional effect as genetic redundancy in populations of organisms and tends to normalize the observed statistical distribution, then one might predict that the genetic instability of cancerous tissue would create a scaling effect that causes pleomorphy in cells and scaling in cell cluster aggregations. Statistical comparison of cell and cell cluster size distributions in normal and cancerous tissues may provide a useful and general screening technique for

detecting when genetic redundancy is compromised by cancer in normal tissues.

Conclusion

Until now, the basis of fluctuating asymmetry has been addressed only with abstract models of hypothetical cell signaling, or elsewhere, at the level of selection working on the organism with potential mechanism remaining in the black box. However, fluctuating asymmetry must first and foremost be envisioned as a stochastic process occurring during tissue growth, or in other words, occurring in an exponentially expanding population of cells. As demonstrated in the model presented here, this expansion process can be represented by stochastic proportional (geometric) growth that is terminated or observed randomly over time. These results imply that the fluctuating asymmetry observed in populations is not only related to potential environmental stressors, but also to a large degree, the underlying genetic variability in those molecular processes that control the termination of growth. Therefore, fluctuating asymmetry responses to stress may be hard to interpret without controlling for genetic redundancy in the population. Both the simulation and experimental results suggest that measures of distributional shape like kurtosis, scaling exponent and tail weight may actually be a strong signal of variability in the underlying process that causes developmental instability. Therefore, the kurtosis parameter of the fluctuating asymmetry distribution may provide more information about fluctuating asymmetry response than does a populations' average or mean fluctuating asymmetry. This may provide a novel method by which to resolve conflicts in previous under-sampled research without the collection of more data.

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Appendix A: Landmarks used for morphometric analyses of fluctuating asymmetry in *Drosophila*

Appendix B: A description of Reed and Jorgensen's generative model of double Pareto-lognormal size distribution

Reed and Jorgensen's (2004) generative model begins with the Itô stochastic differential Equation (B1) representing a geometric Brownian motion given below.

$$dX = \mu X dt + \sigma X dw \quad (\text{B1})$$

with initial state $X(0) = X_0$ distributed lognormally, $\log X_0 \approx N(v, \tau^2)$. After T time units the state $X(T)$ is also distributed lognormally with $\log X(T) \approx N(v + (\mu - \sigma^2/2)T, \tau^2 + \sigma^2 T)$. The time T , at which the process is observed, is distributed with density $f_T(t) = \lambda e^{-\lambda t}$, where λ is a constant rate. The double Pareto-lognormal distribution (B2) is generated when geometric Brownian motion is sampled repeatedly at time t with a negative exponential probability (Figure 1).

$$f(x) = \frac{1}{x} \cdot g(\log(x)) \quad (\text{B2})$$

given the normal-Laplace distribution

$$g(y) = \frac{\alpha \cdot \beta}{\alpha + \beta} \cdot \phi\left(\frac{y - v}{\tau}\right) \cdot \left[R\left(\alpha \cdot \tau - \frac{y - v}{\tau}\right) + R\left(\beta \cdot \tau + \frac{y - v}{\tau}\right) \right]$$

where the Mill's ratio $R(z)$ is

$$R(z) = \frac{1 - \Phi(z)}{\phi(z)}$$

and where Φ is the cumulative density function and ϕ is probability density function for standard normal distribution $N(0,1)$, where α and β are parameters that control power-law scaling in the tails of the lognormal distribution.

The limiting forms of the double Pareto lognormal are the left Pareto lognormal ($\alpha = \infty$), right Pareto-lognormal distributions ($\beta = \infty$), and lognormal distributions ($\alpha = \infty$, $\beta = \infty$) with Pareto tails on only the left side, only the right side, or on neither side, respectively.