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The Symmetry of Longevity

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Abstract

We tested the hypothesis that relatively symmetrical flies live longer. Vein measurements on the left and right wings from the same individual were used to characterize bilateral symmetry in relationship to age-specific survival in defined cohorts. A longitudinal cohort study supported the hypothesis of a correlation between bilateral symmetry and longevity. For another type of experiment, wings were removed from females and males at approximately the beginning of adult life. Thus, there would be no effect of wings per se on adult survival. These wings were measured to characterize bilateral asymmetry, and the day of death of each dewinged individual was determined. Wing symmetry of females and males proved to be a statistically significant predictor of life span, especially for males.

It has been argued that chance events in complex biological processes can substantially affect longevity (1,2). This hypothesis is motivated by the observation that genetically identical individuals living in the same environment can exhibit major differences in life span (1). Development is a complex process that is a likely source of chance events that could affect life span. Developmental error, or instability, has often been invoked as the basis of subtle bilateral asymmetries in animals (3). This asymmetry is called “fluctuating” when the average trait deviation in a population is 0 (there is no net left-side or right-side bias) and the distribution of deviations is symmetric around 0. There is an extensive literature on the subject (4) that includes the use of fluctuating asymmetry as an index of fitness, performance, developmental precision, and developmental stability (5–7). In this regard, other indexes of asymmetry (in addition to fluctuating asymmetry) are prospectively useful (8). Using *Drosophila melanogaster* in demographic studies, we tested the hypothesis that morphological asymmetry is inversely related to life span. We found that more symmetrical *D. melanogaster* lived longer.

Two experiments were conducted on wing measurements in relation to age-specific survival. The first was a longitudinal experiment based on naturally occurring death in a large cohort. Wings were measured from females that died at different ages. In the first experiment, wing measurement asymmetry was statistically significantly negatively associated with female longevity in the population cage. In the second experiment, wings from males and females were removed early in adult life. Individuals were then housed separately, and the day of death was recorded. In the second experiment, we found that the measure of wing asymmetry was a statistically significant predictor of life span of both sexes and the relationship was stronger for males than for females. Increased asymmetry was associated with reduced longevity.

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Methods

Flies from a long-established laboratory population (out-bred, wildtype) of *D. melanogaster* were used for both experiments reported in this study. The population was initiated from at least 100 isofemale lines established from females collected at the Ravenswood winery, Sonoma, California (9), and maintained in bottle culture in the laboratory at a population size of at least 1000 of each generation. For experiments, flies were reared on a standard *Drosophila* diet under constant illumination at 21°C. For the population experiment, approximately 10,000 newly eclosed flies (females and males) were released in a large population cage with periodically replaced fresh fly food. The cage was monitored until all flies died. Every 3 days all the females that died in a defined 4-hour period were collected and frozen at -20°C for a longitudinal experiment on female reproductive characteristics extrapolating from Carlson and Harshman (10). Using wings from a random sample of these females, we discovered that wing asymmetry was associated with longevity in the population cage. We conducted a second experiment in which the morphological structures (wings) were removed at an early age. For the dewinged fly experiment, males and females were held together at a consistent density in bottles with food until they were 2–3 days old. At this time, the flies were anesthetized by ether followed by wing removal near the base using fine forceps and scissors. The forceps were not used to grasp the body to reduce the chance of decreased viability from handling. Approximately 275 wingless flies of each sex were held individually at 28°C under constant illumination in vials that were replaced every 3 days with fresh food.

In experiment 1, we recorded a fly as dead and then proceeded to measure wing characteristics at that time. In this experiment, wing characteristics could have changed over time as a function of age. Wing asymmetry at death is the response, and age-at-death is the predictor. In experiment 2, asymmetry was the predictor and age-at-death was the response, as the wings were measured first (just after eclosion) and age-at-death was recorded as the response.

For both experiments, both wings were measured from each fly using a microscope and ocular micrometer. Three measures were made on each wing: The length of the posterior crossvein (100× magnification) and wing vein landmark measurements represented width and length (20× magnification) as described previously (11). In the population cage experiment all wings were measured by two investigators. The correlation coefficient of the measures by two individuals was 0.9258 ($p < .0001$). However, one of the data sets was missing measurements of one wing vein, thus only the complete data set was used. For both experiments, the wings were measured blind with respect to sample identity.

In this article, we define two measures of an individual's asymmetry based on wing measurements. The first measure is asymmetry distance, which measures how far away given data are from symmetry, and is defined as $d = \|x - Px\|$. Here, Px is the orthogonal projection of individual measurement vectors $x = (W_1, L_1, C_1, W_2, L_2, C_2)$ on the linear subspace corresponding to symmetry, defined by $W_1 = W_2$ (left and right width coincide), $L_1 = L_2$ (left and right length coincide), and $C_1 = C_2$ (left and right crossvein coincide). Note that symmetry corresponds to $d = 0$, and as d increases, so does asymmetry. A second measure of individual asymmetry is the asymmetry angle $\alpha = \arccos(\|Px\|/\|x\|)$. This quantity corresponds to the composite angle between the vectors of the actual measurements x and the projected vectors Px (as defined above). A smaller angle indicates increased symmetry, with an asymmetry angle $\alpha = 0$ corresponding to the symmetric case.

For regressing measures of wing asymmetry on age-at-death in the analysis of the first experiment, we used least squares regression. For the analysis of the second experiment, where we regress age-at-death on wing asymmetry measures, it was first observed that variance of age-at-death was roughly proportional to the mean. To adequately reflect this

heteroscedasticity of the response data in the statistical model, we applied Poisson regression with the canonical log-link function for regressing age-at-death on wing asymmetry measures [with the goal to adapt to the quasi-Poisson mean-variance structure, noting that from the quasi-likelihood perspective, the data do not have to be counts or have a Poisson distribution as a prerequisite for applying a Poisson regression model (12)]. The data were also analyzed using a standard measure of fluctuating asymmetry as used by Woods and colleagues (11). We summed over the three wing measurements, obtaining $f = \sum_i |L_i - R_i| / \text{mean}(|L_i - R_i|)$, where (L_i, R_i) , $i = 1, 2, 3$, are the (left, right) measurements of the wing width, length, crossvein, respectively, and where $\text{mean}(|L_i - R_i|)$ is the arithmetic mean of the $(|L_i - R_i|)$, $i = 1, 2, 3$.

Because it was still possible that the asymptotic distributions would not provide correct approximations for distributions of the parameter estimates in the Poisson regression model, we also performed a bootstrap test (13). When creating bootstrap samples, we resampled independently from the (sex, age-at-death) pairs along with the asymmetry angle and the fluctuating asymmetry measure independently, under the null hypothesis of no relationship between age-at-death and asymmetry angle. We ran the bootstrap by taking all measurements from an individual, if that individual was sampled in the bootstrap resampling procedure with replacement, creating bootstrap samples of the same size as the original sample of flies. This was a nonparametric bootstrap procedure which did not presume anything about the nature of the regression relationship and is commonly used for bootstrapping in regression analysis.

Data from both studies was screened for leverage points and outliers. One leverage point in the second study was removed before statistical analyses. In addition, we fit a linear model by robust regression (14,15). The goal of this additional analysis was to address any problems with positive constraints leading to non-normal errors, and the effect of outliers and leverage points, all of which may invalidate inference for least squares methods that assume normality. We did all of the statistical analysis in R. The robust regression analysis was implemented by function `rlm` in the MASS library downloaded from R (<http://www.r-project.org>).

Results

Experiment 1

In the population cage experiment (experiment 1; Table 1 presents summary statistics), the correlation between the composite asymmetry measure defined by asymmetry angle and female life span was statistically significant ($p < .0001$). Similarly, there were statistically significant correlations between longevity and separate wing measurements of length ($p < .0001$) and width ($p < .0001$), but not the crossvein. Three methods were used to test the effect of the error term; in no case was there a significant relationship between error and asymmetry, and the relationship between lifetime and asymmetry remained significant apart from any temporal trends in the error. A standard measure of fluctuating asymmetry summed over the three wing vein measures (11) also had a statistically significant association with life span ($p < .0001$).

For experiment 1, two models were used to analyze the data: Model 1A (Figure 1A) was a linear model with age-at-death as the predictor and asymmetry distance as the response. The fitted model was:

$$\text{Asymmetry distance} \sim 0.032 - 0.0009 \text{ Age} - \text{at} - \text{death},$$

with standard error (*SE*) of 0.0002 (t value = -4.6, $p < .0001$, correlation $r = -0.409$) for the slope of the regression line, indicating that for those flies with higher age-at-death we observed less asymmetry.

Model 1B (Figure 1B) was the same as Model 1A, but with asymmetry angle as the response. The fitted model was:

Asymmetry angle $\sim 0.013 - 0.0004$ Age – at – death,

with *SE* of 0.0001 (t value = -4.67 , $p < .0001$, correlation $r = -0.412$) for the slope of the regression line. Model 1B also indicates that for those flies with higher age-at-death, we observed less asymmetry.

The results of the robust regression analysis of experiment 1 are as follows. The fitted model was:

Asymmetry distance $\sim 0.028 - 0.0005$ Age – at – death,

with *SE* of 0.0001 (t value = -4.03 , $p < .0001$, correlation $r = -0.409$) for the slope of the regression line, indicating that for those flies with higher age-at-death, we observed less asymmetry. For the asymmetry angle as the response, the fitted model was:

Asymmetry angle $\sim 0.0112 - 0.0002$ Age – at – death,

with *SE* of 0.0001 (t value = -4.087 , $p < .0001$, correlation $r = -0.412$) for the slope of the regression line. The results of the robust regression are comparable with the non-robust analysis, except for the smaller slope estimates. For those flies with higher age-at-death, we observed less asymmetry.

Experiment 2

In the experiment in which dewinged flies were housed separately (experiment 2; Table 2 presents summary statistics), wing bilateral asymmetry as measured by asymmetry angle was a statistically significant predictor of life span ($p < .0001$). In this model, wing asymmetry is measured first and it does not change during the life span since the wings were removed. Age-at-death is then the response.

Prediction equations were generated from the Poisson regression analysis: male age-at-death $\sim \exp(3.73 - 14.84 \text{ asymmetry angle})$, female age-at-death $\sim \exp(3.73 - 3.31 \text{ asymmetry angle})$. Both models provided highly significant fits. We also fitted a Poisson regression model that combined all data for both sexes and included an interaction term (Asymmetry angle \times Sex) between these predictors. The model was:

Age – at – death $\sim \exp[3.73 - 14.84 \text{ Asymmetry angle} - 0.005 \text{ Sex} + 11.53 (\text{Asymmetry angle} \times \text{Sex})]$,

where Sex=0 for males and Sex=1 for females. The analysis showed that all parameters were significant with the exception of sex ($p = .87$); however, the interaction of sex with asymmetry angle was significant ($p < .01$). Both male and female slopes were significantly different from 0, and they were significantly different from each other. There was evidence for a statistically significant effect of asymmetry angle on age-at-death ($p < .0001$) and a significant interaction between the effects of sex and asymmetry angle ($p < .01$). The analysis showed that males are more vulnerable to the life-span-decreasing effect of asymmetry. A standard measure of fluctuating asymmetry, used by Woods and colleagues (11), was also used to analyze the data from dewinged flies, and this analysis led to similar results (data not shown).

Figure 2A shows the scatter plot of the data for 193 females with the outlier identified. Removal of this leverage point made no difference in the outcome of the statistical analysis. Figure 2B shows the fitted model for 192 females, and Figure 2C shows the fitted model for 213 males. Both slopes are significant and they differ from each other. Asymmetry is more strongly associated with shortened male life span than with female life span. We found significant improvement by adding the predictors to the model.

A nonparametric bootstrap analysis was also conducted to assess the significance for fluctuating asymmetry in relation to age-at-death, and also for asymmetry angle in relation to age-at-death. For fluctuating asymmetry, there were 976 bootstrap samples (of 1000) in which the fitted coefficients for fluctuating asymmetry were greater than the fitted coefficient observed for the original data (-0.02489). For the asymmetry angle, there were 997 bootstrap samples (of 1000) for which the fitted coefficients of the asymmetry angle as a predictor for age-at-death was greater than the fitted coefficient observed for the original data (-14.8376). Bootstrap analyses thus confirmed that there was a significant relationship between age-at-death and both fluctuating asymmetry and asymmetry angle ($p < .05$).

The results of the robust regression analysis of experiment 2 are as follows. A linear model by robust regression is also fitted. Prediction equations were generated from the robust regression analysis for males and females separately: male age-at-death $\sim 41.75 - 621.90$ asymmetry angle with SE of 294.30 (t value = -2.113 , $p < .036$, correlation $r = -0.133$), female age-at-death $\sim 42.13 - 124.92$ asymmetry angle with SE of 326.71 (t value = -0.3824 , $p > .10$, correlation = -0.031). As can be seen, the model for the male was moderately significant at the .05 level, and the model for the female was not significant. We also fitted a linear robust regression model that combined all data for both sexes and included an interaction term (Asymmetry angle \times Sex) between these predictors. The model was:

$$[\text{Age} - \text{at} - \text{death} \sim 41.72 - 610.33 \text{ Asymmetry angle} + 0.546 \text{ Sex} \\ + 487.09 (\text{Asymmetry angle} \times \text{Sex})],$$

where Sex = 0 for males and Sex = 1 for females. The analysis showed that only the coefficient of the asymmetry angle was significant (t value = -2.0026 , $p < .05$).

Discussion

Our results indicate a positive association between morphological symmetry and longevity. This was the case for both experiments and all analyses including the nonparametric bootstrap, although the robust regression, which is likely to have less power against the alternative of interest in our analysis, indicated that in experiment 2 only the effect for males was moderately significant and that only the coefficient of asymmetry was significant when the data from males and females were combined.

Other studies have documented a positive association between symmetry and fitness characters (4,16). A meta-analysis of 29 studies found that symmetry was a consistent predictor of fitness accounting for a relatively small proportion of the variation: 2.1% of growth, 12.3% of fecundity, and 6% of survival. Our results are similar in that they are consistent for two quite different experiments and account for approximately 5% of the variation in age-at-death. It has been noted that composite measures of symmetry, such as the measures introduced in this article, are relatively powerful and relatively uncommon (4). The present study observed that asymmetric males were relatively frail; this frailty may be a function of deleterious alleles at hemizygous genes on the X chromosome. An effect of sex chromosomes on asymmetry has been implicated in earlier studies (4). Our study possesses unique features in that: (a) in experiment 2, the morphological trait characterized for symmetry was decoupled from the second trait (age-at-death, life span) because the wings were removed and age-at-death was determined for dewinged flies and (b) the wings were removed at an early adult age which strongly implicates the pre-adult stage as the source of the association between survival and symmetry. Chance events during development are believed to be the source of asymmetry (4); our study provides the strongest experimental evidence that this is the case.

We introduced two measures of asymmetry at the individual level, based on the notion of comparing a given individual with the closest fictitious perfectly symmetric individual. We

project the measurements of a given individual on this symmetric proxy, and measure the distance between a given individual and the symmetric proxy by either the length of the projection or by the angle between actual and projected measurements. The asymmetry measures derived from this concept have proven useful in the present study. They might be of interest for future studies of asymmetry to focus on symmetry at the individual level.

Considerable research has been conducted to identify biomarkers of aging and longevity, but it has been difficult to find whole organism characters that have predictive value. In one of our experiments, wing asymmetry was a statistically significant predictor of life span even though wings were removed soon after the beginning of the adult stage (long before death). Thus, wing asymmetry was probably an indicator of events earlier in life that are correlated with life span. The present study provides unique evidence that early life events contribute to an association between longevity and asymmetry. To clarify this phenomenon, it will be important to study the genetics and developmental biology that underlie bilateral asymmetry (3,17), perhaps with a focus on relevant molecular mechanisms such as transcription factors that underlie bilateral differentiation during development (18). In terms of interpreting specific results from the present study, the presence of a single copy of the X chromosome in *Drosophila* males is known to result in exposure to recessive deleterious genes; this result suggests a mechanism for increased male frailty and developmental instability. Our results have implications for understanding human longevity including insight into life-span differences between males (relatively short-lived) and females, the correlation between handedness and longevity, and the relationship between fetal environment and longevity (19). Finally, it might be possible to use anatomical measurements, such as facial characteristics, to partially predict human longevity. Our work has general implications and suggests further study of the relationship between form, development, and life span.

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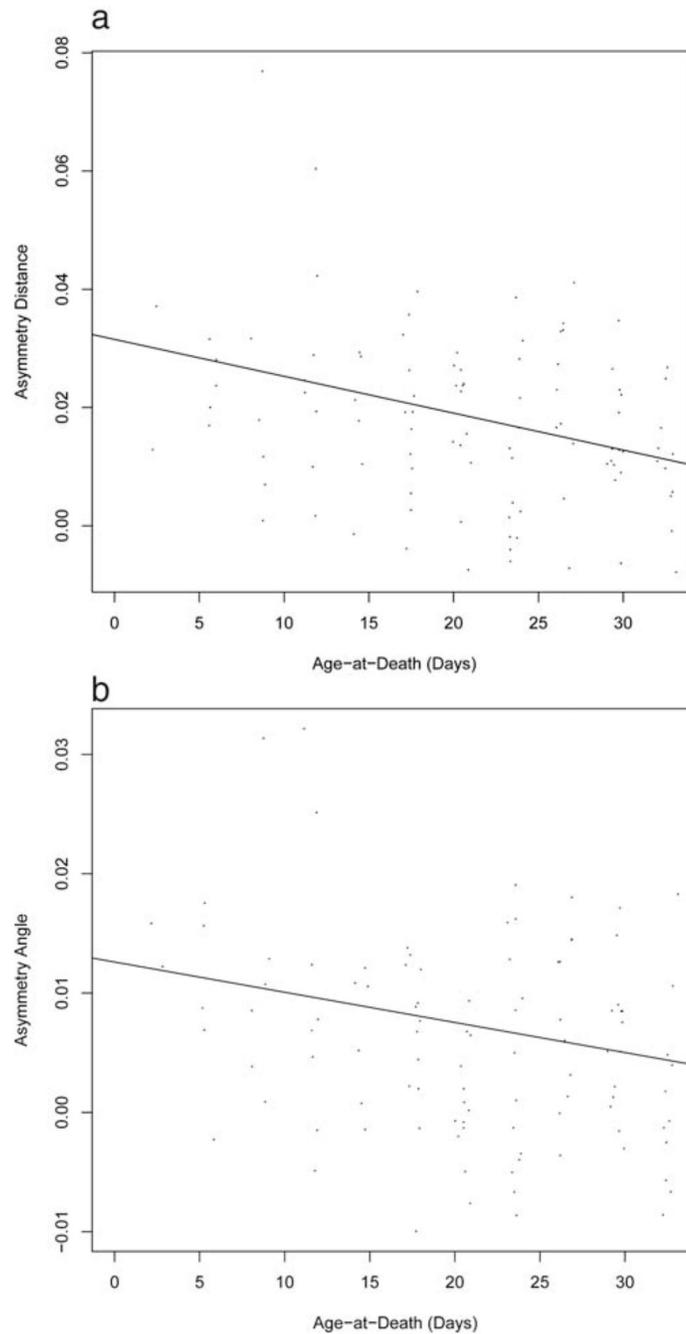


Figure 1.

A, Simple linear model for experiment 1: Age-at-death as the predictor, our new asymmetry distance as the response. The figure depicts the fitted line [Asymmetry distance $\sim 0.032 - 0.0009$ Age-at-death ($p < .00001$, correlation $r = -0.409$)] overlaid with the scatter plot. The regression line shows that Asymmetry distance decreases as Age-at-death increases. **B**, Simple linear model for experiment 1: Age-at-death as the predictor, our new asymmetry angle as the response. The figure depicts the fitted line [Asymmetry angle $\sim 0.013 - 0.0004$ Age-at-death ($p < .0001$, correlation $r = -0.412$)] overlaid with the scatter plot. The regression line also suggests that Asymmetry angle decreases as Age-at-death increases.

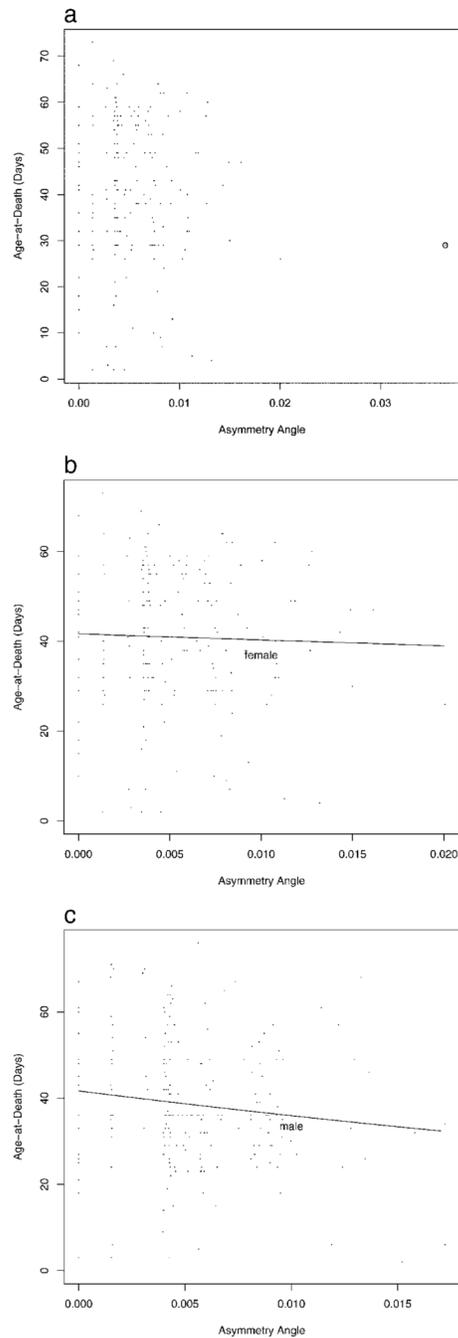


Figure 2.

A, Scatter plot of Age-at-death versus Asymmetry angle for 193 female flies in experiment 2, with one outlier circled. **B**, Poisson regression model for 192 female flies (with the outlier left out) in experiment 2: Asymmetry angle as the predictor, Age-at-death as the response, with the canonical log-link function. The fitted model [predicted female Age-at-death $\sim \exp(3.73 - 3.32 \text{ Asymmetry angle})$] is overlaid with the scatter plot of the 192 female flies. The predicted female age-at-death is a decreasing function of asymmetry angle. **C**, Poisson regression model for 213 male flies in experiment 2: Asymmetry angle as the predictor, age-at-death as the response, with the canonical log-link function. The fitted model [predicted male Age-at-death

$\sim \exp(3.73 - 14.84 \text{ Asymmetry angle})$] is overlaid with the scatter plot of the 213 male flies. The predicted male age-at-death is also a decreasing function of asymmetry angle.

Table 1
Summary Statistics for Experiment 1 ($N = 108$ Females)

Variable	Mean	<i>SD</i>
Age at death	21.564	8.578
Asymmetry distance	0.0181	0.0131
Asymmetry angle	0.0071	0.0053
Length (left wing)	1.4762	0.0443
Width (left wing)	1.0049	0.0302
Crossvein (left wing)	0.1805	0.0101
Length (right wing)	1.4762	0.0466
Width (right wing)	1.0073	0.0294
Crossvein (right wing)	0.1814	0.0102

Notes: Females were collected on the day of death in a population cage for wing-vein measurements to test the hypothesis that adult life span is associated with wing asymmetry.

SD = standard deviation.

Table 2
Summary Statistics for Experiment 2 ($N = 193$ Females, 213 Males)

Variable	Females		Males	
	Mean	SD	Mean	SD
Age at death	40.699	15.501	38.600	14.695
Asymmetry distance	0.0138	0.0104	0.0118	0.0078
Asymmetry angle	0.0054	0.0042	0.0052	0.0035
Length (left wing)	1.4939	0.0397	1.3153	0.0368
Width (left wing)	1.0006	0.0326	0.8951	0.0317
Crossvein (left wing)	0.1808	0.0114	0.1532	0.0103
Length (right wing)	1.4940	0.0408	1.3170	0.0370
Width (right wing)	1.0033	0.0306	0.8977	0.0311
Crossvein (right wing)	0.1816	0.0107	0.1538	0.0102

Notes: Dewinged females and males were housed individually in vials where the day of death was determined to test the hypothesis that wing asymmetry predicts longevity.

SD = standard deviation.